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MOSAIC AND STREAK DISEASES OF ROSE[1]

By PHILIP BRIERLEY, *pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry,* and FLOYD F. SMITH, *entomologist, Division of Truck Crop and Garden Insect Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture*[2]

INTRODUCTION

Rose mosaic, or infectious chlorosis, was first described by White (*19*)[3] from New Jersey and Massachusetts in 1928. The disease was considered as serious and widespread in the United States in later reports by White (*1, 20, 21, 22, 23*),[4] Nelson (*13*), and Weiss and McWhorter,[5] but its importance was discounted by Milbrath (*11, 12*). Field evidence suggesting natural spread was advanced by McWhorter.[6] Other chlorotic disorders, particularly in rose understocks, were evidently confused with rose mosaic in many of these early surveys. Some of the early concern over rose mosaic was due to claims that affected plants might be killed. Immature Manetti stocks (*25*) were responsible for outright loss of some plants in the year in which mosaic was first recognized. It is probable that the effects of immaturity were confused with mosaic in some early observations, since typical rose mosaic is rarely if ever fatal.

The present study was undertaken to determine whether insect vectors of rose mosaic occur (1932–35) and to devise means of control (1933–38). As the work progressed it became necessary to redefine the symptoms of the disease (*4*), excluding certain types of symptoms attributed to rose mosaic by others but not found transmissible to hybrid tea roses in the writers' tests. A new estimate of prevalence has been made by indexing samples of understocks and by testing transmissibility of chlorotic disorders appearing naturally in roses. Yellow variants of the mosaic pattern were encountered, and also an apparently undescribed virus disease called "streak" (*3*) because of its necrotic effects in certain rose varieties. At present rose mosaic is attracting attention in California, and streak in Texas. A discussion of the relation of nursery practices to spread of these diseases has already appeared (*5*).

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[2] The writers are indebted to a number of nurserymen for supplying samples of commercial understocks, and to Freeman Weiss, senior pathologist in the Division of Mycology and Disease Survey, Bureau of Plant Industry, for samples of rose mosaic and a collection of stocks affected with "crinkle" assembled prior to 1933.

[3] Italic numbers in parentheses refer to Literature Cited, p. 659.

[4] WHITE, R. P. AN INFECTIOUS CHLOROSIS OF ROSES. U. S. Dept. Agr. Plant Dis. Rptr. 12: 33–34. 1928. [Mimeographed.]

[5] WEISS, FREEMAN, and MCWHORTER, FRANK P. PACIFIC COAST SURVEY FOR ROSE MOSAIC. U. S. Dept. Agr. Plant Dis. Rptr. 14: 203–205. 1930. [Mimeographed.]

[6] MCWHORTER, FRANK P. FURTHER REPORT ON ROSE MOSAIC IN OREGON. U. S. Dept. Agr. Plant Dis. Rptr. 15: 1–3. 1931. [Mimeographed.]

The writers' type stock of rose mosaic was a plant of Mme. Butterfly and its vegetative offspring. This plant was obtained from a New Jersey greenhouse, and the symptoms were confirmed as typical at the time by White. The stock of healthy Mme. Butterfly came from a greenhouse in Washington, D. C., where it had long been propagated on its own roots. No mosaic was detected in the source stock and no natural infection has appeared in 6 years' experimental culture of the writers' material.

SYMPTOMS

TYPICAL ROSE MOSAIC

The symptoms of typical rose mosaic in the Mme. Butterfly variety are essentially as described by White (*23*), who originally defined the disease. In the course of 6 years' growth under glass and in the field, the writers' type mosaic affected Mme. Butterfly has developed symptoms of several patterns. The typical symptoms are prominent chlorotic areas commonly adjacent to and feathering away from the midrib and accompanied by more or less severe distortion of the leaflet (fig. 1, *A;* also *23, fig. 1, A, B*). These patterns are hereinafter called "typical symptoms." Such chlorotic areas may appear in each leaflet of a leaf, or one or more leaflets may remain symptomless. Not infrequently a whole leaf on an affected shoot may fail to express symptoms, but buds taken from the axils of such symptomless leaves have proved infectious.

A second symptom expressed in the same plant line consists of more or less clearly defined rings, erratic wavy lines or watermark types, and oak-leaf patterns (fig. 1, *D*). White has illustrated this symptom type (*23, figs. 2, G; 3, C; 4, E*), and Valleau's (*18, fig. 26*) rose virus patterns are also of this order. Old leaves that have developed in the greenhouse during the summer months sometimes have shown extensive grayish-yellow areas with green islands (fig. 1, *E*). Cuttings from the type mosaic-affected Mme. Butterfly grown in the field at the Arlington Experiment Farm, Arlington, Va., showed striking yellow vein-banding patterns in May 1934 (fig. 1, *H*). This symptom was expressed only in known mosaic-affected plants and was absent from healthy plants of Mme. Butterfly of the same age that had been grown adjacent to the affected plants.

All the mosaic symptoms in Mme. Butterfly described above have appeared in a single plant line. No evidence of mixture of two or more viruses in this line has been detected in a large number of inoculations to various types of roses and subinoculations back to Mme. Butterfly (see table 5). It is therefore assumed, in the absence of any evidence to the contrary, that the several symptom types mentioned above are responses to infection with one virus, namely, the rose mosaic of White.

The symptoms described for Mme. Butterfly include nearly all the types known to be expressions of typical rose mosaic in the genus *Rosa*. In Ophelia, Radiance, Rapture, and Templar, symptoms are essentially the same as in Mme. Butterfly, the chlorotic patching about the midribs predominating. In Briarcliff, Columbia (fig. 1, *C*), George C. Waud, Odorata (*Rosa odorata* Sweet, P. I. 22994; fig. 1, *B*), Talisman, and The Queen, symptoms are similar but less conspicuously developed. The watermark type and the field expression of prominent yellow

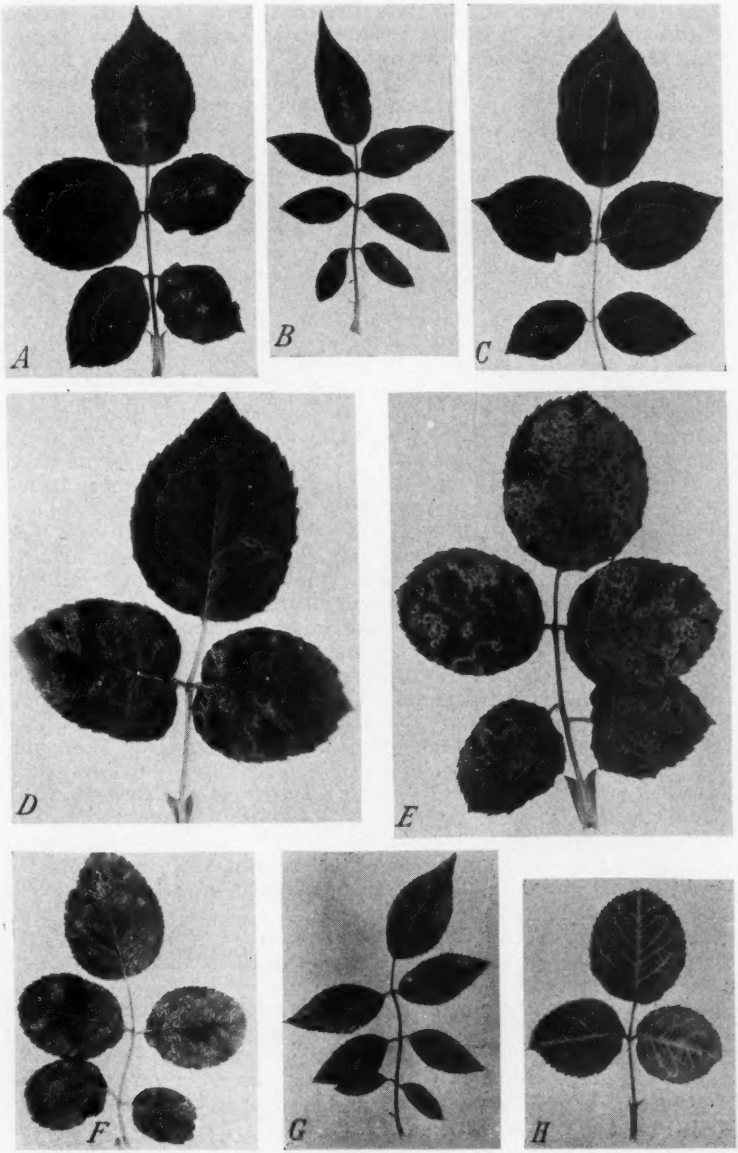

FIGURE 1.—Symptoms of typical rose mosaic. *A*, Characteristic symptoms in Mme. Butterfly; *B*, in Odorata; *C*, in Columbia; *D*, ring and watermark patterns in Mme. Butterfly; *E*, dull-yellow blotching with green islands in Mme. Butterfly; *F*, watermark pattern in Paul's Scarlet Climber; *G*, dull-yellow watermark pattern in field-grown Odorata; *H*, yellow vein banding in field-grown Mme. Butterfly. *C* and *F*, Experimentally produced; others, natural infections.

vein banding have appeared in Briarcliff also. In Blaze, Conrad Ferdinand Meyer, Duchess of Wellington, Joanna Hill, Kaiserin Auguste Viktoria, Mme. Berthe Fontaine, Mrs. A. R. Waddell, Mrs. John Laing, Paul's Scarlet Climber (fig. 1, *F*), *R. hugonis* Hemsl., *R. nutkana* Presl., *R. wichuraiana* Crép., Souvenir de Claudius Pernet, and Ulrich Brunner, only the oak-leaf-watermark-ring symptoms have been observed. Subinoculation from mosaic-affected plants of the varieties named to healthy Mme. Butterfly always produced symptoms comparable to those in the type plants of affected Mme. Butterfly.

Mosaic has been experimentally produced in the understocks Canina (*Rosa canina* L.), Manetti (a variety of *R. noisettiana* Thory), Multiflora (*R. multiflora* Thunb.), Odorata, Ragged Robin (Gloire des Rosomanes), and Texas Wax (*R. multiflora*×*R. chinensis*). Natural infection has been demonstrated in Manetti, Odorata, and Ragged Robin. In Manetti the symptoms include chlorotic patching about the midrib and also watermark patterns (fig. 2, *B*). Expression of these symptoms is the exception rather than the rule under greenhouse conditions, even in plants proved to be mosaic-affected. For example, in 28 healthy Manetti plants budded to known mosaic hybrid teas during April and May 1934 and grown continuously in the greenhouse, mosaic symptoms were first recognizable in 3 plants on April 9, 1935, nearly 12 months after inoculation. Of the remaining 25 plants, 1 showed mosaic symptoms on June 19, 4 more on July 19, 3 on August 7, and 1 on September 6, 1935. In all, 12 of the 28 plants in this trial showed evidence of mosaic within 16 months after budding. All buds lived and developed into shoots, and all these scion shoots expressed symptoms. In general, the more vigorously growing Manetti plants were more likely to express symptoms. No resistance is believed to be involved here, but merely irregular expression of symptoms. When cuttings were rooted from symptomless plants thus inoculated and buds from healthy Mme. Butterfly were set in these rooted cuttings, they developed into typical mosaic-affected shoots. Similarly, buds from the symptomless Manetti induced mosaic in Mme. Butterfly plants when set in the canes of that variety. Manetti is thus shown capable of masking mosaic under greenhouse conditions.

Diagnosis of mosaic in Manetti in the field is even less trustworthy and is complicated by the frequent occurrence of the crinkle or "rattlesnake" symptoms (fig. 2, *C*, *D*, *E*), which are not transmissible to hybrid tea roses but are more conspicuous than true rose mosaic symptoms in Manetti. White's illustrations of mosaic in Manetti (*23, fig. 1, D, E*) correspond more closely to the crinkle type than to rose mosaic symptoms.

Odorata develops chlorosis and puckering near the midrib or on the larger branch veins (fig. 1, *B*). This symptom is similar to that typically expressed by Briarcliff. The pattern is developed both under glass and in the field with more constancy than are symptoms in Manetti. Watermark patterns may also occur (*23, fig. 2, G*). In the field a dull-yellow watermark has appeared (fig. 1, *G*) in mosaic-affected Odorata.

Rosa multiflora develops ring patterns or watermark types, the prevailing pattern varying with the strain of this understock. Both the Japonica and the Chenault strains of *R. multiflora* have expressed symptoms regularly in the greenhouse, and diagnosis from symptoms expressed in the field has proved fairly accurate. The speckle type

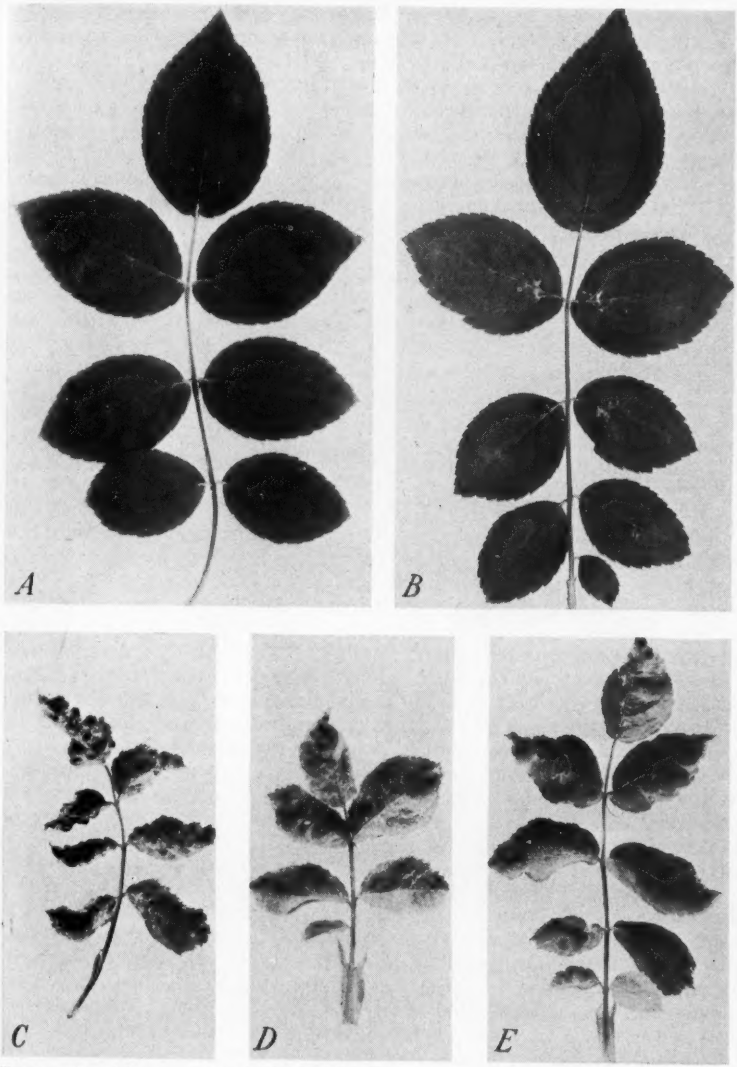

FIGURE 2.—Mosaic and crinkle in Manetti. *A*, Healthy specimen; *B*, mosaic-affected specimen from greenhouse, showing faint watermark patterns as well as typical mosaic symptoms; *C*, *D*, *E*, crinkle or "rattlesnake" patterns in Oregon field-grown Manetti. Natural infections. (Photograph by F. P. McWhorter.)

(see fig. 8, *A;* also *23, fig. 2, B, E*) has not appeared consistently in Multiflora plants budded to mosaic, and when appearing naturally has not proved transmissible to Mme. Butterfly.

Ragged Robin, when experimentally infected, showed inconspicuous watermark patterns in a few leaves and occasional weak expressions of typical symptoms. The chlorotic speckle pattern common in Ragged Robin has not proved transmissible to Mme. Butterfly. The weak expression of mosaic symptoms in this variety and the prevalence of a distinct and apparently innocuous speckle present problems of diagnosis closely comparable to those in Manetti.

Texas Wax developed occasional well-defined watermark patterns after bud inoculation. Experimentally infected plants, when transferred to the field, showed a dull-yellow watermark similar to that expressed by Odorata. Marked crinkling and dwarfing of leaflets on vigorous young canes, common in Texas Wax in the vicinity of Washington, has not been found transmissible to hybrid tea roses.

The type symptoms of rose mosaic remain clearly defined in leaves of Mme. Butterfly and Odorata that were pressed 5 years ago. Likewise oak-leaf patterns in C. F. Meyer and George C. Waud are readily recognizable after a like interval.

YELLOW MOSAICS[7]

Five collections of mosaic from naturally affected roses differed from the typical rose mosaic in symptoms. The sources of the yellow types were Talisman from Rockville, Md.; C. F. Meyer from New York, N. Y.; Margaret McGredy from West Grove, Pa.; and Irish Charm and an unnamed hybrid perpetual rose from gardens at the Arlington Experiment Farm, Arlington, Va. The Rockville collection was from a greenhouse; all others were from the field. In each instance a single affected plant was found among many plants free from mosaic symptoms. The symptoms expressed in the original affected plants and on transfer to Mme. Butterfly differ from the typical rose mosaic chiefly in color. The chlorotic areas are in general a brighter and lighter yellow than in typical mosaic[8] and are often extensively developed and very conspicuous. In most varieties and most strains there is less tendency to puckering of the leaves in the yellow types, but one of the Arlington farm collections may produce marked puckering.

Comparison of the five yellow mosaic collections in Briarcliff, Mme. Butterfly, C. F. Meyer, Margaret McGredy, and Talisman over a period of a year or more showed differences among the yellow types. No two of the five yellow variants were alike in symptom expression in all five varieties. The observed differences were of the order commonly used in distinguishing strains of a virus. The Talisman strain induced bright conspicuous symptoms in most varieties (fig. 3, *A, B*), and was distinct from typical rose mosaic in all varieties except Paul's Scarlet Climber. In Briarcliff it often produced bright-yellow blotching in young canes. In one Multiflora seedling it produced tan streaks and patches in the bark of young canes, dwarfing of both

[7] Thomas and Massey (*17*) distinguish rose mosaics 1, 2, and 3. Their rose mosaic 1 is evidently White's rose mosaic (the writers' "typical rose mosaic"). Their rose mosaics 2 and 3 are similar to those the writers have termed "yellow mosaics."

[8] L. C. C. Krieger made the following matched-color readings in Maerz and Paul (*10*). Yellow mosaic (Talisman strain) in Briarcliff leaves: M. and P. 10K2 (specimen not quite as bright); normal leaf about M. and P. 23L8. Typical mosaic in Briarcliff leaves: M. and P. 22 between K and L6; normal leaf M. and P. 23L7, a little greener. Typical mosaic in Mme. Butterfly leaf: M. and P. 22L6; normal leaf M. and P. 23L between 7 and 8. Dull-yellow pattern of typical mosaic in old leaves of Mme. Butterfly: M. and P. 2013, specimen a trifle grayer; normal leaf near M. and P. 23L7.

leaves and shoots, and dieback, in addition to yellow watermark patterns. Tan cane lesions developed in *Rosa nutkana* also. The C. F.

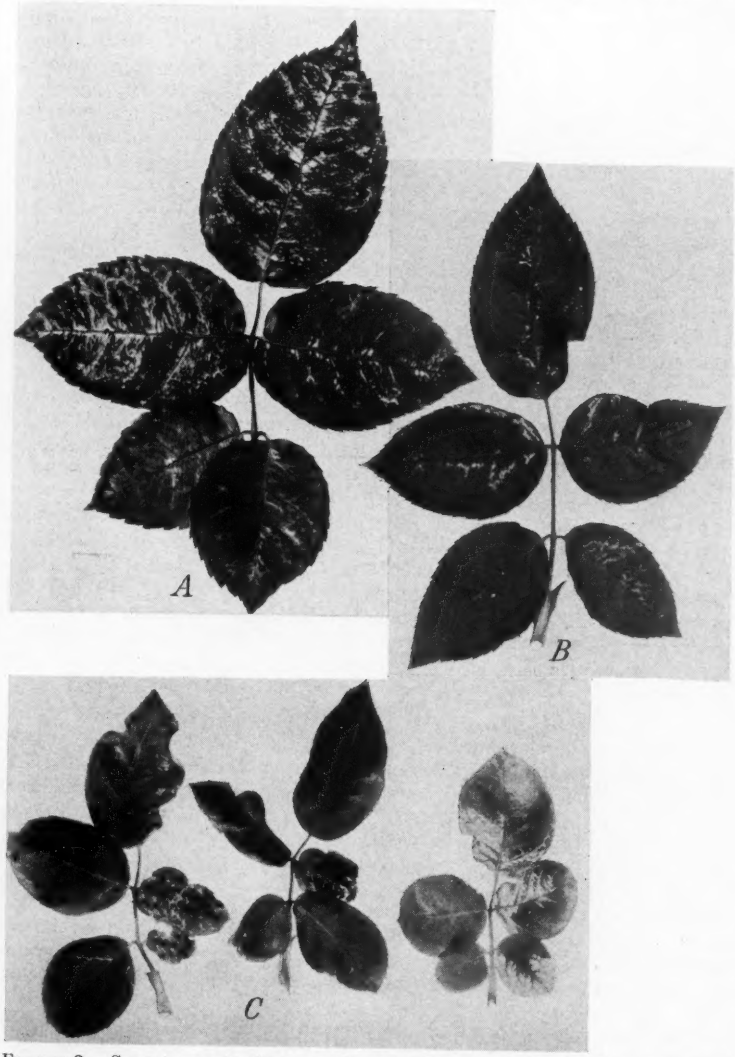

FIGURE 3.—Symptoms of yellow mosaics: *A*, Talisman strain in Mme. Butterfly; *B*, Talisman strain in Briarcliff; *C*, one of two Arlington farm strains in the source plant, an unnamed hybrid perpetual variety. *A* and *B*, Experimentally produced infections; specimens from greenhouse; *C*, natural infections, specimens from field.

Meyer strain expressed distinctive virus patterns in Briarcliff, C. F. Meyer, Mme. Butterfly, and Margaret McGredy. The Irish Charm

strain developed watermark patterns in C. F. Meyer, Margaret McGredy, and Talisman, but no distinct evidence of infection in the other two test varieties. In the source plant it developed prominent yellow blotching and ring markings without distortion of the leaflets.

The Margaret McGredy strain was recognizable as a yellow variant of rose mosaic in all test varieties. The strain from an unnamed hybrid perpetual rose from the Arlington farm (fig. 3, *C*) gave strikingly conspicuous symptoms in the source plant. In the test varieties it was hardly recognizable as a virus disease except for inconspicuous watermarking in Briarcliff and necrotic effects in Talisman. In Talisman it behaved much like rose streak, involving necrosis of the bud followed by general dieback and death of the plant. Failure to produce streak effects in Briarcliff and Mme. Butterfly distinguishes the strain from the streak disease, however. This test was repeated three times, and in each of the four trials Talisman died, with similar symptoms.

Differentiation of strains in the rose mosaic group is of no particular importance and there is no assurance that they are strains of one virus. The tendency of some well-defined yellow mosaics to revert to crinkle symptoms in certain varieties is, however, interesting. In view of this behavior it would not be surprising to find that some of the crinkle symptoms in hybrid perpetuals (see fig. 8, *B*) represent transmissible types, although the writers have not been able to demonstrate it. Evidently Briarcliff and Mme. Butterfly are not particularly responsive as test varieties for possible viruses of the crinkle type.

White's "albication of Golden Ophelia" appears from his illustration (*23, fig. 3, E*) to be a characteristic yellow mosaic. Milbrath's "albication of Souvenir de Claudius Pernet" (*12, fig. 126*) also appears to belong to this group, showing distinct oak-leaf margins between the yellowed and green leaf areas. The writers have been unable to get material of either of these earlier reported yellow mosaics for comparison.

ROSE STREAK

Rose streak disease, which previously has been described briefly (*2*), was first noticed at the Arlington farm in the late summer of 1933. It has since been found at Beltsville, Md., Washington, D. C., and New York, N. Y. Characteristic symptoms were noted at the Arlington farm in October 1934, in 60 varieties of rose, including teas, hybrid teas, hybrid perpetuals, hybrid multifloras, hybrid wichuraianas, hybrid rugosas, hybrid Bengals, Noisettes, Chinas, and polyanthas. Specimens submitted from Texas by J. J. Taubenhaus and E. W. Lyle showed typical streak symptoms. Subinoculation from Lyle's specimens of the Edel variety, showing ring symptoms in both canes and leaves, produced the characteristic symptoms of this disease in Mme. Butterfly.

Symptoms of three classes have proved reliable for diagnosis of streak: (1) Brownish rings (fig. 4, *A*) and brown vein banding (fig. 4, *B*) in fully expanded leaves, usually accompanied by brownish or greenish, often water-soaked, ring patterns in canes (fig. 5) (the cane symptoms have been reproduced in experimentally produced streak infections in Odorata, Multiflora, and Silver Moon under glass, but they are less conspicuous and the coloring is more dilute than in the field); (2) green senescence designs similar to the brown patterns (fig. 4, *B, C*), which are often expressed in leaves that are later pre-

maturely abcised; and (3) a yellowish-green vein banding (fig. 4, *D*) in certain hybrid multiflora and hybrid wichuraiana roses. This symptom is usually accompanied by greenish water-soaked rings or dull-

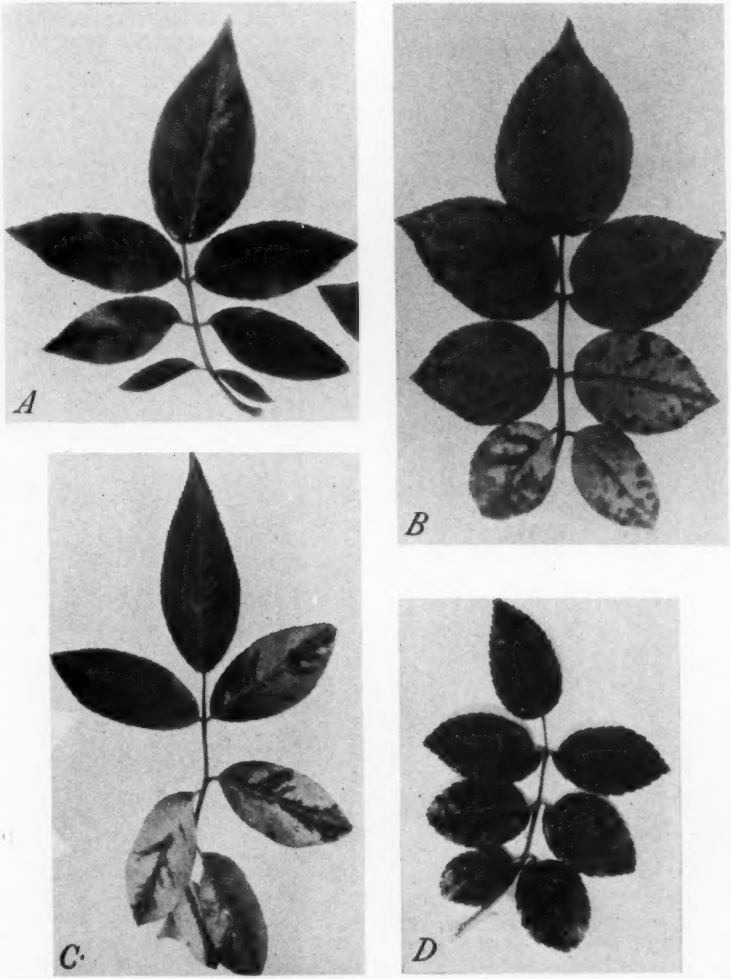

FIGURE 4.—Leaf symptoms of rose streak: *A*, Brown rings in Odorata; *B*, discontinuous brown vein banding and senescence patterns in Van Fleet Hybrid No. 36; *C*, senescence patterns in Odorata; *D*, yellowish-green vein banding in Jean Girin. All natural infections; specimens from the field.

brownish rings in the canes (fig. 5). White's figures of "veinal chlorosis" (*23, fig. 5, B, C, D*) strongly resemble this streak symptom, but White did not report cane symptoms. A plant of the hybrid rugosa rose Sarah Van Fleet, showing a bright-yellow mottling in

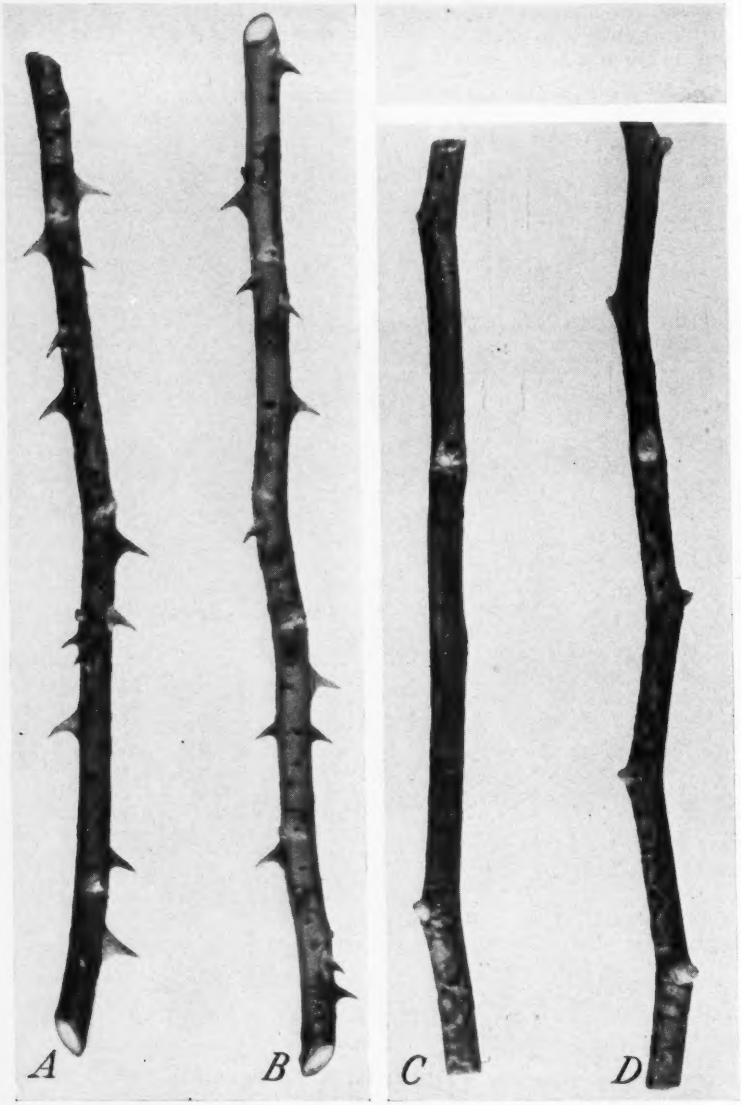

FIGURE 5.—Symptoms of rose streak disease in rose canes: *A* and *B*, Canes of Jean Girin; *C* and *D*, canes of Miss G. Mesman. All natural infections; specimens from the field, Arlington, Va.

leaves but no cane lesions, yielded only streak on subinoculation to Mme. Butterfly. The rugosa group seems the most difficult of the common garden types in which to diagnose streak, but some varieties show well-defined cane symptoms when affected.

In addition to these streak symptoms found in affected roses in nature, an experimentally produced symptom deserves mention. When streak-affected buds are set in canes of certain hybrid tea roses, such as Briarcliff, Mme. Butterfly, Ophelia, and others, the stock turns nearly black [9] and necrotic (fig. 6) about the inserted bud soon after union has been established.

Sometimes nearly black, necrotic secondary lesions appear on young lateral branches arising below the inserted bud (fig. 7, *A*). Commonly

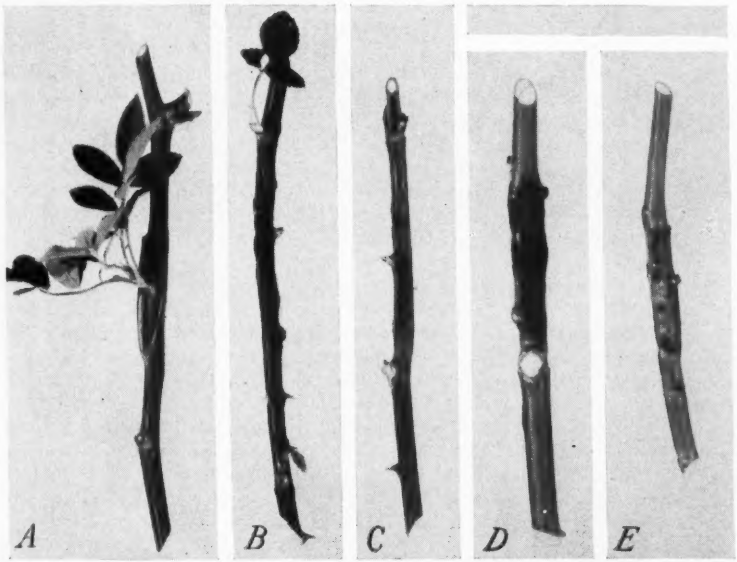

FIGURE 6.—Primary necrotic lesions of rose streak in Mme. Butterfly and Briarcliff. *A*, *B*, *C*, Mme. Butterfly canes budded with Odorata November 6, 1934: Odorata is healthy in *A*, streak-affected in *B* and *C*. *D*, *E*, Briarcliff: *D*, Budded with streak-affected Miss G. Mesman October 5, 1934; *E*, budded with streak-affected Silver Moon December 5, 1934. Photographed January 31, 1935.

the stem is girdled at the site of the bud, and the distal parts die and the leaves wither but persist (fig. 7, *B*). This is a necrotic primary lesion in the cane, and the virus is capable of limited further movement under favorable conditions. Such effects have not been recognized in nature, but if natural spread of the streak virus were taking place, comparable but perhaps smaller primary lesions of this type should appear in varieties of this group.

[9] L. C. C. Krieger made the following matched-color readings based on Maerz and Paul (*10*) and on Ridgway (*14*). Streak in Mme. Butterfly cane. M. and P. 56L1 (specimen very much darker); Ridgway 13M, maroon (specimen very much darker). Streak in Multiflora canes, M. and P. 56L1 (specimen darker); Ridgway 13M, maroon but with more black in it. Streak in *R. wichuraiana* canes, M. and P. 6L10 to a rifle lighter upwards on the cane; Ridgway 15K, morocco red (specimen a little lighter).

FIGURE 7.—Symptoms of rose streak in Mme. Butterfly (6-inch pots): *A*, Budded from streak-affected American Pillar July 15, 1934. Note necrosis about the bud and also four secondary necrotic lesions on cane at left. The leaf at base was shed from top of left cane. *B*, Budded from streak-affected Multiflora September 2, 1933. The Multiflora bud has been cut away from cane at right; the lesion has advanced downward, involving the lateral at right, on which withered leaves persist. Cane at left remains unaffected. Photographed August 30, 1934.

Multiflora bud has been cut away from cane at right; the lesion has advanced downward, involving the lateral at right, on which withered leaves persist. Cane at left remains unaffected. Photographed August 30, 1934.

VIRUSLIKE PATTERNS NOT FOUND TO BE TRANSMISSIBLE

Various crinkle types, often accompanied by dwarfing and twisting of leaflets and whole leaves, have proved nontransmissible to hybrid teas in trials by the writers. Most important is the crinkle type in Manetti, known in the Northwest as "rattlesnake" (fig. 2, *C, D, E*). Mild symptoms consist of yellowish-green speckling without distortion or dwarfing of the leaves; the spots are small and angular and may be very densely distributed without consistent association with the veinlets. A more severe expression involves crinkling and distortion of the leaflets, especially at the tips. The most striking expression of this series of symptoms includes marked dwarfing of the leaflet, puckering throughout, and frequently twisting of the petiole.

Odorata and Texas Wax often display conspicuous dwarfing, puckering, and twisting of leaves in zones a foot or more in length on vigorous canes. Although chlorotic expressions are less evident in these stocks, the symptoms mentioned appear under similar conditions and seem to belong to the same series as the Manetti symptoms described. A misalinement of leaflets without dwarfing is a milder expression commonly appearing at either end of a zone of severe symptoms. In Multiflora the faulty alinement of leaflets on petioles and twisting of petioles also occur under conditions that permit development of "rattlesnake" in Manetti and its counterparts in Odorata and Texas Wax. Chlorosis and dwarfing do not commonly accompany this twisting in Multiflora.

Attempts have been made to correlate the crinkle type in Manetti, Texas Wax, and Odorata with environmental factors. In the spring of 1934 Manetti from two sources was brought at intervals from cold storage into a cold propagating house. Crinkle or "rattlesnake" was very prevalent in March, but plants moved to a warmer greenhouse developed normal new foliage. Later plantings of similar stock showed milder crinkle in the propagating house. A lot of Manetti, removed from cold storage on March 23, developed normally in a warm greenhouse but expressed some crinkle in a cool house and in an outdoor frame. Field expression of the crinkle type has shown a marked tendency to show zonation characterized by series of crinkled leaves alternating with series of normal leaves on the same cane. This suggests a controlling environmental factor that fluctuates periodically, such as temperature, light intensity, or moisture. However, no clear relation with such environmental factors was detected in a preliminary study of the development of zonation in field Manetti.

Manetti, Texas Wax, and Odorata stocks all showed a marked tendency to develop crinkle when grown under cheesecloth shade on low land at the Arlington farm. Manetti of Oregon, California, and English origin showed crinkle August 14, 1934, 6 weeks after being moved to this site. Texas Wax, Odorata, and Odorata seedlings similarly expressed crinkle in the new growth produced after establishment on this shaded site. However, cloth houses on a hillside at Beltsville, Md., failed to induce typical crinkle. Shade is evidently not the sole determining factor in the expression of this symptom.

Interest in the factors that determine the expression of crinkle declined with the accumulation of evidence that this symptom is unrelated to rose mosaic. Healthy Mme. Butterfly budded into the Manetti stocks mentioned here and into other lots with crinkle history developed into mosaic-free plants. Crinkle might be the expression

of a virus disease that the writers have failed to detect because the test plants used (chiefly Briarcliff and Mme. Butterfly) were not suited to its expression. If such is the case, the expression of symptoms in Manetti is very sensitive to environment, and the virus is exceedingly widespread in rose stocks. Until some evidence of virus etiology is advanced, the writers prefer to interpret the trouble as a direct environmental effect. The original interest in crinkle or

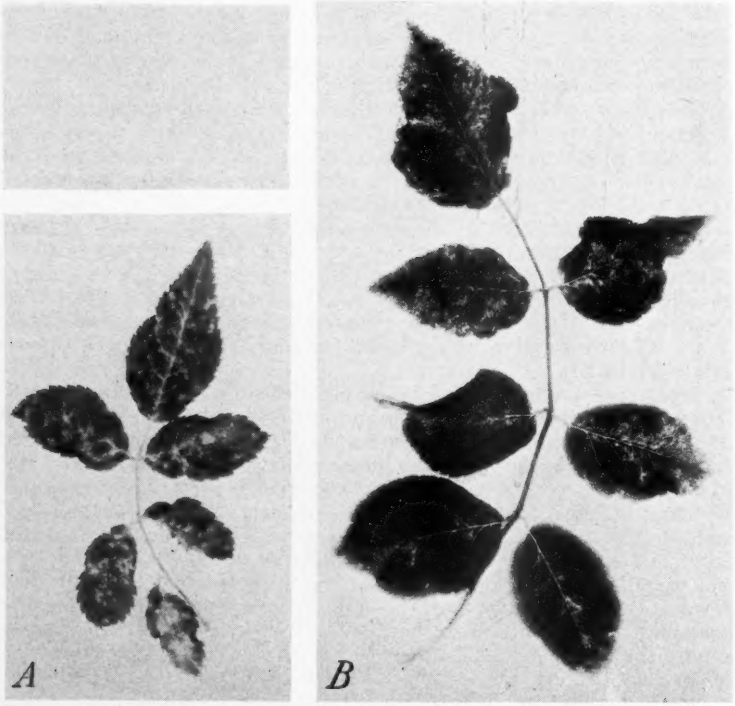

FIGURE 8.—Symptoms suggestive of virus diseases but not found transmissible to hybrid tea roses: *A*, "Speckle" type in Multiflora from greenhouse; *B*, mosaiclike pattern in unnamed hybrid perpetual rose from field.

"rattlesnake" is attributable to three factors: (1) The symptoms suggest virus etiology; (2) investigators observed crinkle appearing in Manetti as a sequence to mosaic inoculation, or observed mosaic appearing in roses worked on crinkled stocks; and (3) surveys, conducted in advance of adequate research, revealed that crinkle was common where mosaic was believed to be common. The second of these factors may now be explained. Since in Manetti crinkle is more common and more easily diagnosed than mosaic, one would expect mosaic to occur occasionally in crinkled Manetti, but no high correlation should appear between crinkle and mosaic content. Again, if mosaic is budded into Manetti the true symptoms (fig. 2, *B*) are usually inconspicuous and irregularly expressed, but crinkle

patterns (fig. 2, *C*, *D*, *E*) may appear whenever the environmental conditions are suitable.

The commercial importance of crinkle types in rose understocks depends on the effect of this peculiarity on their performance as understocks. If crinkled Manetti is as well suited as symptomless Manetti for use as a stock for hybrid tea roses, then crinkle is of technical interest only. No critical test of this point has been made, but growers' trials have shown no conspicuous difference in performance of crinkled as compared with symptomless Manetti. The writers' experience has brought to light no adverse effects in hybrid tea roses worked on stocks with crinkle history, but a carefully designed experiment would be necessary to determine whether there is any detectable difference in merit as stocks between crinkled and normal-appearing Manetti.

A speckle type in Multiflora has appeared sporadically in the field and under glass in the writers' material (fig. 8, *A*). Affected leaves are thickly sprinkled with angular chlorotic spots of varying size. The leaflets are often dwarfed but do not show the characteristic puckering associated with crinkle or "rattlesnake." Speckle is typically expressed under glass, but "rattlesnake" is largely suppressed and only the milder phases become evident under the writers' greenhouse conditions. Thirty-seven of 329 seedlings raised from speckled Multiflora parents showed speckle in turn. This symptom appeared in seedlings grown continuously under glass as well as in those grown in the open. Ragged Robin may also develop a similar chlorotic spotting under glass and in the field.

TABLE 1.—*Rose species and varieties showing symptoms suggestive of virus disease but producing no mosaic symptoms in Briarcliff or Mme. Butterfly when budded into one or both varieties*

Source plant	Source locality	Symptoms in source plant
Briarcliff	Arlington, Va	Speckle.
Do	Rockville, Md	Do.
Dr. Eckener	West Grove, Pa	Do.
Julia Countess D'Ortig	Vineland, Ont	Crinkle.
Mme. Berthe Fontaine	Arlington, Va	Do.
Manetti	do	Do.
Do	Doylestown, Pa	Do.
Do	Hillsboro, Oreg	Crinkle (fig. 2, *C*, *D*, *E*).
Mrs. F. R. Pierson	Arlington, Va	Speckle, striped flower.
Mrs. John Laing	Vineland, Ont	Crinkle.
Odorata	Arlington, Va	Do.
Do	do	Albication.
Ragged Robin	do	Speckle.
Rosa alberti Reg	Beltsville, Md	Crinkle.
R. spinosissima altaica Rehd	do	Do.
R. canina L	Arlington, Va	Albication.
R. multiflora Thunb	do	Crinkle.
Do	do	Speckle (fig. 8, *A*).
R. nutkana Presl	Hillsboro, Oreg	Do.
R. pisocarpa A. Gr	Beltsville, Md	Crinkle.
R. rugosa Thunb	Madison, Wis	Yellow mottle.
R. rugosa Thunb. var. *heterophylla* Hort.	West Grove, Pa	Crinkle.
R. villosa L	Beltsville, Md	Yellow crinkle.
R. webbiana Wall	do	Yellow mottle.
R. willmottiae Hemsl	West Grove, Pa	Speckle, vein clearing.
R. xanthina Lindl	New York, N. Y	Speckle.
Striped La France	Arlington, Va	Crinkle.
Texas Wax	do	Do.
The Queen	Madison, N. J	Speckle.
Ulrich Brunner	Ithaca, N. Y	Do.
White Maman Cochet	Arlington, Va	Crinkle.
York and Lancaster	West Grove, Pa	Speckle, striped flower.
Unnamed	Beltsville, Md	Crinkle.
Do	do	Vein clearing.
Unnamed (6 varieties)	Arlington, Va	Crinkle (fig. 8, *B*).

Speckle and crinkle symptoms occur in hybrid tea and hybrid perpetual roses in the field and less commonly and less prominently in the greenhouse. A number of rose species and varieties showing such symptoms are listed in table 1. None of the source types listed there have induced distinctive symptoms in healthy Briarcliff or Mme. Butterfly. Although this is certainly not conclusive evidence that these symptoms do not represent virus diseases, it is convincing proof that they are not symptoms of rose mosaic. As suggested in the section on yellow mosaics, some of the crinkle patterns in hybrid perpetuals may prove to be virus diseases.

EXPRESSION OF SYMPTOMS IN THE FIELD

In 1934 roses of 5 varieties, experimentally infected with mosaic in the greenhouse, were grown in the field at the Arlington farm to determine the reliability of symptom expression in known mosaic-affected material. The plants were examined at intervals for recognizable mosaic symptoms. During the season 12 of 20 Briarcliff plants, 27 of 27 Mme. Butterfly, 3 of 4 Canina, and 5 of 5 Ophelia expressed symptoms sufficiently distinct for reliable diagnosis, and one mosaic-affected Duchess of Wellington failed to do so. In the same season 4 of 5 mosaic-affected Canina in a cloth house developed distinctive oak-leaf and watermark patterns.

In 1935, a similar experiment was set up at Beltsville, Md., and observed through 1936 and in part through 1937. The observations on expression of rose mosaic are summarized in table 2. The number of plants observed in this experiment changed as individuals died and as additional plants were set from time to time. The number showing symptoms varied as new leaves developed and old leaves were lost. Thus a plant might show symptoms at one reading and none at the next.

In a parallel trial the Talisman strain of yellow mosaic expressed recognizable symptoms in single plants of Briarcliff, Canina, Joanna Hill, Mme. Butterfly, Multiflora, Odorata, and Texas Wax with great regularity; in Manetti symptoms were recognizable on two of the five dates of observation; in Ragged Robin symptoms were not recognized. In a further parallel trial, single plants inoculated with buds from streak-affected Silver Moon were observed through three seasons in the field. Texas Wax expressed streak symptoms at three dates out of five; Multiflora showed symptoms once; Canina, Joanna Hill, Manetti, and Ragged Robin remained symptomless throughout the period of observation.

It is clear from these records that symptom expression is uncertain in plants known to be infected with mosaic. Symptoms of the Talisman yellow strain are more regularly expressed, but still may be lacking in Manetti and Ragged Robin. Streak expression is erratic even in Texas Wax and Multiflora, while no expression of symptoms was detected in Canina and Manetti, which are susceptible to systemic invasion. The performance of Joanna Hill and Ragged Robin has less bearing on the question of symptom expression since these varieties have not been shown to be susceptible to streak. Diagnosis of the diseases in these trials was hampered by slow growth of the plants during dry weather and by loss of leaves from leaf diseases at times. Such conditions may be encountered in commercial rose culture also,

and the trials are thus probably representative of what can be expected in the diagnosis of mosaic and streak in rose nurseries.

TABLE 2.—*Symptom expression of rose mosaic in the field at Beltsville, Md., in plants experimentally infected when set in March 1935, or later*

Variety	June 7, 1935		Aug. 21, 1935		Oct. 12, 1935		Sept. 9, 1936		Oct. 30, 1937	
	Plants observed	Plants affected	Plants observed	Plants affected	Plants observed	Plants affected	Plants observed	Plants affected	Plants observed	Plants affected
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Briarcliff	3	1	2	1	3	3	2	2		
Canina	3	3	3	2	3	2	3	3	3	1
Joanna Hill	1	0	3	0	3	0	2	1		
Mme. Butterfly	3	3	3	2	3	3	2	2		
Manetti	3	0	3	2	3	0	3	1	3	0
Multiflora	1	0	2	1	2	2	2	1	2	2
Odorata	3	0	3	3	3	3	3	0		
Ragged Robin	1	1	1	1	1	1	1	1		
Texas Wax	3	1	3	3	3	0	3	3	3	0

DISTRIBUTION AND PREVALENCE

Naturally occurring rose mosaic from 20 sources has been confirmed by transfer to healthy Mme. Butterfly. These sources include 17 varieties of roses and rose stocks (Blaze, C. F. Meyer, Duchess of Wellington, Georg Arends, George C. Waud, Irish Charm, Joanna Hill, Laurent Carle, Mme. Butterfly, Manetti, Margaret McGredy, Mrs. F. R. Pierson, Odorata, Ragged Robin, Talisman, Templar, and one unnamed). Seven States (New York, New Jersey, Pennsylvania, Maryland, Virginia, Oregon, California) and the Province of Ontario were represented. Five of the 20 collections were yellow types, which consistently differed in symptoms from the typical mosaic; the remaining 15 sources were indistinguishable from the type on transfer to Mme. Butterfly. This evidence supports the general view that rose mosaic is widespread. Because of the systemic nature of the disease and the general shipment of roses and rose understocks, mosaic may be expected to appear sporadically throughout this country and elsewhere. The writers' samples are believed to be representative of rose mosaic in the United States and Canada, and except for the yellow variants there is no evidence that the type of mosaic varies from one section to another.

Previous survey records of the prevalence of rose mosaic are now open to question, since crinkle and speckle symptoms were previously accepted as characteristic of rose mosaic. In the field the more common understocks, Manetti, Multiflora, Odorata, Ragged Robin, and Texas Wax, all commonly express symptoms which suggest virus patterns and which were formerly accepted as evidence of rose infection. For example, in a collection of supposed mosaic-affected stocks assembled at the Arlington farm prior to 1933, indexing by means of Briarcliff and Mme. Butterfly buds revealed 1 mosaic-affected plant in 89 Manetti, 1 in 21 Odorata, and no mosaic present in 27 Multiflora or in 14 Texas Wax plants (table 3). It was therefore thought desirable to index samples of commercial understocks to determine the percentage of typical rose mosaic naturally occurring. Indexing was carried out either by rooting the cuttings under test and budding them to healthy Mme. Butterfly (fig. 9) or budding from the

FIGURE 9.—Healthy Mme. Butterfly budded on Manetti to test for presence of mosaic in this stock: *A* is affected with mosaic; *B* and *C* are normal. Budded June 4, 1934, in 6-inch pots. Photographed August 30, 1934.

test stocks into canes of healthy Mme. Butterfly. Index tests were kept under observation for several months, or longer than was required for expression of symptoms following transfer of known mosaic-affected buds under comparable conditions. The period necessary for expression of symptoms varied with the growth rate of the plants under test and with other factors.

The results of indexing understocks are shown in table 3. No mosaic was found in 103 Texas Wax and 27 Multiflora plants. One of 21 plants of Odorata proved to be affected, and 3 Ragged Robin plants of 208 had mosaic. Two large lots of Manetti proved to be 2.9 and 12.8 percent affected, and two others were less than 1 percent affected. In addition to the Manetti tabulated (table 3), 1 of 55 Joanna Hill plants and 5 of 31 Templar plants, received from a California nursery as "dormant buds" in Manetti, showed mosaic when these buds expanded. No mosaic was found in samples of the Shafter, Calif., stock of 1933 after it had grown for 2 years at Babylon, N. Y., Hillsboro, Oreg., or Portland, Oreg. No evidence appeared from these tests to indicate that typical rose mosaic spreads in the field in Oregon or New York in the absence of budding and grafting operations. The prevalence of mosaic in understocks is somewhat lower than has been claimed, but still high enough to permit extensive spread by indiscriminate budding. The yellow mosaic types have been found as isolated plants only, as previously mentioned. No yellow types were found in the understocks indexed.

TABLE 3.—*Prevalence of rose mosaic in understocks as determined by budding to the Mme. Butterfly variety*

Stock	Source	Source year	Plants indexed	Plants showing mosaic
			Number	*Number*
Manetti	England	1929	17	0
Do	do	1930	24	0
Do	Hillsboro, Oreg	1930	14	1
Do	do	1931	8	0
Do	do	1933	202	1
Do	Portland, Oreg	1930	2	0
Do	do	1931	16	0
Do	Irvington, Calif	1935	86	11
Do	Montebello, Calif	1935	105	3
Do	Shafter, Calif	1932	28	0
Do	do	1933	156	1
Do	Shafter, 1933; Babylon, N. Y. (2 years)	1935	36	0
Do	Shafter, 1933; Hillsboro, Oreg. (2 years)	1935	63	0
Do	Shafter, 1933; Portland, Oreg. (2 years)	1935	47	0
Do	Miscellaneous		8	0
Multiflora	Texas	1930	7	0
Do	Arlington, Va	1931	20	0
Odorata	Hillsboro, Oreg	1930	6	0
Do	Burlingame, Calif	1930	6	1
Do	Sumner, Wash	1930	2	0
Do	Arlington, Va	1931	7	0
Ragged Robin	Hemet, Calif	1935	86	1
Do	Montebello, Calif	1930	3	0
Do	Ontario, Calif	1935	74	1
Do	Puente, Calif	1935	41	1
Do	Miscellaneous	1931	4	0
Texas Wax	Scottsville, Tex	1930	14	0
Do	do	1934	89	0

Streak of roses is limited in known distribution to Maryland, New York, Texas, Virginia, and the District of Columbia. The virus from some 30 sources has reacted uniformly, showing no evidence of differentiation into strains. This disease has never appeared in the com-

mercial understocks indexed, but has been demonstrated in Odorata and Multiflora growing at the Arlington farm. Streak was prevalent at the Arlington farm in a collection of rose species and varieties later discarded. Thirty percent infection was recognizable in one collection of varieties said to have been acquired over a period of years from many nurseries. However, the possibility of increase by propagation on affected stocks cannot be entirely excluded. These circumstances suggested natural spread of the disease at the Arlington farm, but spread has not been proved. At Beltsville, Md., 80 healthy Silver Moon plants failed to show any symptoms of streak after 4 years' growth in the midst of a collection of 1,200 roses that included a considerable number of streak-affected plants, some of which were in contact with Silver Moon test plants. Present evidence, therefore, indicates that no natural spread of streak occurs, but the possibility remains that spread may occur in certain seasons or in other localities.

TRANSMISSIBILITY

TRANSMISSION BY BUDDING AND GRAFTING

Typical rose mosaic, yellow mosaics, and streak are readily transmitted to susceptible roses by various methods of budding and grafting, but not by juice inoculation insofar as is known. Inarch grafts, patch grafts, splice buds, and T-buds have all proved effective when union was established. The last-named method has been used most extensively because of its convenience.

The interval between budding and the appearance of the first recognizable symptoms of these diseases is highly variable. During this period union must be accomplished, and the virus must increase and move to a growing point in which young leaves are expanding. Canes are of approximately the same stage of growth when suitable for budding, but the growth of the inserted bud and shifts in shoot dominance after budding are subject to wide variations. Apparently the best circumstances for prompt production of symptoms include the rapid development of a young lateral shoot close below the transferred bud.

The shortest incubation periods recorded by the writers for rose mosaic are 20 days for 1 transfer from Mme. Butterfly to Mme. Butterfly, 22 days once, 26 days 3 times, and a large number of records of 28 to 49 days with other variety combinations. Under unfavorable circumstances, symptoms have been delayed for 12 months or more.

For yellow mosaics the minimum observed period was 17 days for a transfer from C. F. Meyer to Mme. Butterfly, with records of 26 days, 30 days, and many of 40 days for other combinations. Some yellow mosaic transfers were first evident after periods of 6 and 7 months under less favorable conditions.

For streak the shortest recorded period was 18 days in a transfer from Van Fleet No. 46 to Briarcliff. Periods of 27 days were recorded a number of times, and many of 40 days or less. A few instances of delayed expression of streak were observed also. There is no evidence here of important differences that might be helpful in distinguishing or separating the viruses. All appear to have about the same minimum incubation period in a given variety, and in all the period is subject to wide variation according to variety, growth, food movement, and shifts of shoot dominance in the rose used as a test plant.

None of the three types of disease is transmitted if the diseased bud dies or is removed before union with the test plant has taken place. In a series of 18 Briarcliff plants inoculated by budding to Talisman affected with the Talisman strain of yellow mosaic, no transmission resulted in 3 plants from which the inserted mosaic buds were removed after 3 days' contact, and none in 3 plants in which the buds died without forming a union during 16 to 25 days' contact. Twelve plants from which the affected buds were removed after 8 to 25 days all became infected with mosaic. No evidence of union was visible at the end of 4 days. After 8 days cell division was recognizable, and after 12 days the buds were beginning to adhere to the stock. Symptoms appeared 1 month to 5½ months after budding.

In a similar experiment, 12 healthy Mme. Butterfly plants were budded with streak-affected Silver Moon buds, which were removed after intervals of 5, 10, and 15 days or left in place. Streak failed to develop in 3 plants from which the infected buds were removed after 5 days. The 9 plants on which streak-affected buds were left in contact for 10 days or more all developed typical streak lesions in 3 months or less. Cell division and adhesion of the bud to the stock were evident after 10 days' contact. After 6½ months all streak lesions were removed by cuts 4½ inches below the bud scars. No further streak symptoms developed thereafter.

All attempts to transmit mosaic and streak by needle and rubbing methods have failed, but these trials have hardly been sufficiently extensive to be conclusive. No natural infection of mosaic or streak has appeared in the writers' collection of roses in 5 years' observation, although knives and pruning shears have been used on diseased and healthy roses without precautions against contamination.

Seedlings from roses affected with mosaic or streak have never carried these diseases in the writers' trials. Twelve seedlings of *Rosa wichuraiana*, 1 of Silver Moon, 27 of Odorata, and 123 of Multiflora were grown from streak-affected parents, but showed no symptoms of the disease. All the parent plants except Odorata were proved to be affected by budding trials; the Odorata parent was diagnosed as streak-affected from symptoms only. Healthy Silver Moon was budded into 9 of the Multiflora seedlings as a further test for the presence of the streak virus in these seedlings; these Silver Moon buds grew into normal, healthy plants. Small populations of seedlings have been grown from mosaic-affected plants of Mme. Butterfly, but no mosaic has appeared in these. It is highly improbable that either mosaic or streak is seed-borne in roses.

The Chenault strain of Multiflora is said to be the progeny, both by seed and by cuttings, of a single plant still growing at the United States Horticultural Station at Beltsville, Md. The parent plant and its vegetative progeny carry streak, as shown by symptoms and by bud transfer to Mme. Butterfly, Odorata, and Silver Moon; but seedlings from this plant have been uniformly free in the writers' experience. The presence of the streak virus in this importation, and in other introduced specimens of *Rosa multiflora* and *R. saturata* Bak., might suggest the possibility that the virus was imported in these species; but they are said to have been brought in as seeds rather than as cuttings. Again, distribution of affected Multiflora cuttings or of Van Fleet roses worked on such rooted cuttings is a possible factor in the distribution of streak in this country. Regardless of whether these possibilities

represent actual truths, it is obviously wise to rely on seed propagation of understocks unless they are known to be virus-free.

Confirmation of streak infection in a variegated variety of *Rosa wichuraiana* from the Arlington farm made possible a striking demonstration of the contrasting behavior of a genetic variegation and a virus disease above and below a graft union. The variegated and streak-affected *R. wichuraiana*, established as a scion shoot after budding into healthy Odorata, showed both the genetic and the virus patterns above the union, but only the streak was transmitted to the stock. This material is unusually well suited for demonstrating this standard test for transmissibility.

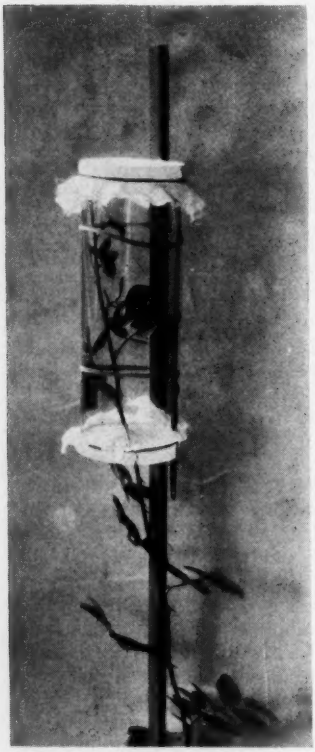

FIGURE 10.—Cage used in insect transmission experiments: The barrel of the cage is of celluloid; the ends are covered with cheesecloth, and the lower end is sewed to make a tight seal, without wrinkles, about the rose cane.

TRANSMISSION EXPERIMENTS WITH INSECTS

Investigations to determine what insects might be capable of transmitting the mosaic and streak diseases of roses were largely exploratory. An effort was made to test all potential vectors, chiefly Homoptera, that occurred on roses or nearby vegetation. Such insect species were obtained by frequent collections in plantings of roses at the Arlington farm, from 1932 to 1935. Collections were also made on survey trips to nurseries and greenhouses in Maryland, Pennsylvania, New Jersey, New York, Ohio, and the District of Columbia.

The number of insects belonging to species capable of breeding on roses was increased by rearing individuals in laboratory cages. Insects belonging to other species died within a short time if confined to rose; therefore tests with such insects were restricted to collected material. All individuals used in experimental tests were preserved and identified by specialists.[10]

In testing for insect transmission, collected or reared insects were confined for 24 hours to several days on tender growths of rose plants infected with either mosaic or streak; they were then transferred to actively growing shoots of healthy plants, where they were permitted to feed until they died, or else for a maximum period of 1 month. A celluloid cage was used to confine insects on terminals of any length

[10] A. C. Mason, Harold Morrison, and P. W. Oman, Division of Insect Identification, Bureau of Entomology and Plant Quarantine.

and was held at any desired height by rubber bands against a stake thrust into the pot of soil (fig. 10). The bottom of the cage was closed by a piece of cheesecloth cut to the center, sewed in place around the stem and thence to the margin, and held in place by rubber bands placed over the branch before putting on the cage. A suction apparatus was employed for introducing or removing insects from the cages, except that aphids were introduced on leaves and removed with a brush. In the case of potted plants all transfers were performed within a transfer chamber to prevent the escape of insects. Most of the experiments were made on potted plants in the greenhouse, but some were conducted on field-grown plants in cheesecloth cages. After all surviving insects had been killed by spraying with pyrethrum extract and the cages had been removed, the plants were maintained in an actively growing condition for periods ranging from 8 to 26 months, so that the development of any disease symptoms might be observed.

The insect species and numbers of individuals used in 229 transmission tests with rose mosaic and in 152 tests with streak are given in table 4. No symptoms indicating transmission of either disease developed in any of the exposed plants. The insects tested included 42 species in the families Cicadellidae, Cercopidae, Membracidae, Araeopidae, Fulgoridae, Aphiidae, and Coccidae, in the order Homoptera, and one species of thrips (order Thysanoptera). As indicated in table 4, a number of species were not tested for transmission of both diseases because these occurred in very small numbers in the areas from which collections were made. It was not considered practicable to rear such less common species in large numbers on their particular, favored hosts for more extensive testing, since it became increasingly evident that natural spread of the two diseases did not occur in the region concerned. The experiments did include, however, all species commonly found on roses in the field or greenhouse and several species that are known vectors of other virus diseases.

In addition to the tests as given in table 4, the following tests were made in an effort to transmit by means of their known vectors recognized viruses from other hosts to the rose variety Mme. Butterfly. In 3 tests, each with 4 adults of *Philaenus leucophthalmus* (L.), strawberry crinkle was not transmitted to rose. In 4 tests with 100 individuals of *Capitophorus fragaefolii* (Ckll.), crinkle was transmitted from strawberry to strawberry, whereas no symptoms developed on 3 rose plants exposed to 75 aphids in parallel tests. In 3 tests with 54 aphids, *Amphorophora rubi* (Kalt.), the vector did not transmit red raspberry mosaic from infected Latham red raspberries to rose; nor was aster yellows transmitted from China aster to 6 rose plants by 75 viruliferous adults of *Macrosteles divisus* (Uhler).

TABLE 4.—*Summary of tests of insects for capacity to transmit the virus of rose mosaic or of rose streak, 1932 to 1935, Arlington, Va.*[1]

Insect tested	Mosaic		Streak	
	Plants exposed	Insects in test	Plants exposed	Insects in test
Family Cicadellidae:	*Number*	*Number*	*Number*	*Number*
Aceratagallia sanguinolenta Prov	2	15	8	53
Agallia constricta Van D	1	12	10	116
Balclutha impicta (Van D.)	2	2		
Carneocephala flaviceps Riley	1	12	5	29
Chlorotettix galbanatus Van D			1	1
Chlorotettix viridius Van D	1	11	5	23
Deltocephalus flavicostus Stål			2	3
Draeculacephala mollipes (Say)	16	95	6	50
Empoasca fabae (Harris)	23	136	6	80
Empoasca maligna (Walsh)	7	110		
Erythroneura sp	8	15		
Exitianus obscurinervis Stål	2	36	3	4
Graphocephala coccinea (Forst.)	2	23	3	6
Graphocephala versuta (Say)			9	96
Gypona octolineata var. *striata* Burm	5	37	2	21
Idiocerus fitchi Van D	1	10		
Kolla bifida (Say)	1	15		
Macrosteles divisus (Uhler)	7	74	4	8
Norvellina seminuda (Say)	2	2	1	1
Oncometopia lateralis (F.)	6	38	4	27
Phlepsia irroratus (Say)	5	32	9	30
Polyamia inimica (Say)	1	16		
Polyamia obtecta (O. and B.)			2	2
Polyamia weedi (Van D.)			1	1
Scaphytopius acutus (Say)	9	60	10	79
Scaphytopius frontalis (Van D.)	1	13	1	14
Stirellus bicolor (Van D.)			2	4
Stirellus obtutus Van D			1	1
Thamnotettix nigrifrons (Forbes)	5	59	8	52
Typhlocyba pomaria McA	7	33	2	25
Family Cercopidae:				
Lepyronia quadrangularis (Say)			1	1
Philaenus leucophthalmus (L.)	48	116	7	21
Family Membracidae:				
Ceresa bubalus (F.)	1	1		
Family Araeopidae:				
Delphacodes puella (Van D.)			1	1
Liburniella ornata Stål			4	13
Family Fulgoridae:				
Scolops sulcipes (Say)			1	1
Family Aphiidae:				
Capitophorus fragaefolii (Ckll.)	3	105	1	25
Macrosiphum rosae (L.)	6	86	9	250
Macrosiphum solanifolii (Ashm.)	32	519	15	255
Myzus porosus Sanderson	13	274	4	120
Family Coccidae:				
Phenacoccus gossypii T. and C	4	38		
Pseudococcus citri Risso	4	82		
Order Thysanoptera:				
Frankliniella tritici (Fitch)			4	40

[1] The source of virus was Mme. Butterfly for rose mosaic, Silver Moon for rose streak; healthy Mme. Butterfly served as test plants. No transmission occurred in any test.

The habits of some of the insect species employed in the above-described transmission tests and the injury they cause on rose are described in a separate publication (*15*).

VARIETAL REACTIONS

All roses adequately tested have been found susceptible to typical rose mosaic (table 5). The characteristic symptom pattern and the severity of the effects vary, but no variety has been found immune. The subinoculations listed in table 5 were made not only to confirm transmission as evidenced by symptom expression but to determine whether the virus could be recovered unchanged. As explained in the discussion of symptoms, several distinct patterns have been ex-

pressed in the type collection of mosaic-affected Mme. Butterfly, including typical rose mosaic, oak-leaf and watermark patterns, dull-yellow rings, and broad yellow vein banding. Duchess of Wellington and Joanna Hill when naturally affected have shown only the oak-leaf-watermark symptoms. When experimentally infected with mosaic from the type in Mme. Butterfly, these varieties and also C. F. Meyer, Kaiserin Auguste Viktoria, Manetti, Paul's Scarlet Climber, Ragged Robin, Silver Moon, Souvenir de Claudius Pernet, Texas Wax, Canina, *Rosa hugonis*, and Ulrich Brunner have developed only or principally these same oak-leaf and watermark designs. On return inoculations to healthy Mme. Butterfly, however, the virus induced the typical symptoms as well as the watermark patterns, and under suitable conditions the dull-yellow rings also. Budding from oak-leaf Duchess of Wellington has induced typical symptoms as well as watermark patterns in Mme. Butterfly in each of five trials and in Ophelia in two trials. Similarly, typical symptoms and watermark have developed in Mme. Butterfly following bud transfer from Souvenir de Claudius Pernet showing sparse and mild watermark patterns in two trials, and in single inoculations from Blaze, George C. Waud, Joanna Hill, Kaiserin Auguste Viktoria, and Texas Wax.

TABLE 5.—*Results of inoculation of rose species and varieties with typical rose mosaic, yellow mosaic (Talisman strain), and streak*

[Affected buds were inserted in the varieties tested, and subinoculations were made as indicated]

Species or variety inoculated	Rose mosaic		Yellow mosaic		Rose streak	
	Symptoms produced	Subinoculations	Symptoms produced	Subinoculations	Symptoms produced	Subinoculations
Briarcliff	+	+	+	+	+	−
Columbia	+	+	+	+	+	
Conrad Ferdinand Meyer	+		+	+	−	
Duchess of Wellington	+	+	+		+	+
Gardenia	+				+	+
Joanna Hill	+	+	+		−	
Kaiserin Auguste Viktoria	+	+	+	+	+	+
Mme. Berthe Fontaine	+					
Mme. Butterfly	+	+	+	+	+	−
Manetti	+	+	+	+	−	+
Margaret McGredy	+		+	+	−	
Mrs. Arthur Robert Waddell	+					
Odorata	+	+	+	+	+	+
Ophelia	+	+	+	+	+	
Paul's Scarlet Climber	+	+	+			
Radiance	+		+		+	
Ragged Robin	+	+	+		−	
Rapture	+	+	+		+	
Silver Moon	+	+	+		+	+
Souvenir de Claudius Pernet	+	+	+	+	−	−
Talisman	+		+	+	+	
Templar	+	+	+		+	
Texas Wax	+	+	+		+	+
The Queen	+					
Rosa canina	+	+	+		+	+
Rosa hugonis	+				−	−
Rosa multiflora	+	+	+	+	+	+
Rosa nutkana	+	+	+		+	+
Rosa wichuraiana	+		+		+	+
Ulrich Brunner	+		+		−	

The milder typical symptoms characteristic of mosaic in Briarcliff, as well as watermark patterns, have been induced in this variety also in a number of inoculations by budding from roses showing only oak-leaf or watermark symptoms. It is apparent that the mild and

inconspicuous symptoms developed in such varieties represent merely varietal responses to typical rose mosaic, since the more conspicuous symptoms can be reproduced from them on transfer to a suitable variety. Varieties reacting like Mme. Butterfly are most severely injured by mosaic. In these the distortion and chlorosis of leaflets are usually conspicuous enough to affect the salability of the cut flowers. Shortening of internodes or general dwarfing of affected plants has not been observed in the writers' pot cultures. The flowers of mosaic-affected plants have not commonly proved defective in the writers' material, although such effects are reported by White [11] and by Nelson (*13*) to occur under conditions of commercial rose culture. It has not been practicable in the writers' work to lay out a critical experiment on comparative performance of healthy and mosaic-affected roses under forcing conditions. In a trial conducted by White (*24*)[12] mosaic-affected plants of the Talisman variety were little if at all inferior to healthy Talisman plants in number of salable flowers or in stem length but a larger proportion of defective blooms were cut from the mosaic-affected plants.

Talisman, Briarcliff, and others of the group developing mosaic symptoms milder than those of Mme. Butterfly, but otherwise similar in pattern, are evidently less injured than Mme. Butterfly. Others, such as Joanna Hill and Duchess of Wellington, which develop symptoms of the ring or watermark type when affected, are so little injured that the disease would be recognized only on close scrutiny.

In the yellow mosaic group the Talisman strain, which alone has been tested extensively, develops prominent leaf symptoms in a larger number of rose varieties than does typical rose mosaic. Cut flowers of affected plants would tend to be of lower sale value because of these conspicuous leaf patterns. Like typical rose mosaic, this yellow type has affected all roses adequately tested.

In the streak disease of roses sharp differences in varietal reaction appear. One group of varieties is subject to systemic infection by the streak virus: e. g., American Pillar, Canina, Clio, Duchess of Wellington, Gardenia, Jean Girin, Kaiserin Auguste Viktoria, Kitchener of Khartoum, Multiflora, Newport Fairy, Odorata, *Rosa nutkana*, *R. wichuraiana*, Sarah Van Fleet, Silver Moon, Texas Wax, Veilchenblau. Such varieties, when affected, display one or more of the characteristic symptoms described above, and any bud suitable for transfer will convey the virus to a susceptible healthy variety. Manetti is evidently allied to this group, inasmuch as systemic infection has been proved by subinoculation, but no characteristic streak symptoms were detected in this understock variety in the writers' trials. Streak has likewise been recovered from symptomless plants of Canina and Multiflora that were slow to express symptoms after inoculation, from Sarah Van Fleet, and from two unnamed roses of the Rugosa type that were naturally infected but symptomless at the time. However, it would be premature to create a subgroup for varieties subject to systemic invasion by the streak virus but showing no symptoms, since symptom expression is highly variable in varieties in which the disease is best known.

A second group of roses is subject to necrotic local lesions only: e. g., Briarcliff and Mme. Butterfly (fig. 6), Columbia, Ophelia, Radiance,

[11] See footnote 4.

[12] NEW JERSEY AGRICULTURAL EXPERIMENT STATION. ROSE MOSAIC NOTES. N. J. Agr. Expt. Sta. Nursery Dis. Notes 5 (11): 2-4. 1933. [Mimeographed.]

Rapture, and Templar. The virus may move a short distance into the stock and induce secondary necrotic lesions in young canes near the inserted streak-affected bud (fig. 7, *A*). After a few weeks or months all affected tissue is dead and the lesions cease to enlarge (fig. 7, *B*).

Subinoculation from any bud suitable for transfer fails to carry the virus to a healthy susceptible test plant. As indicated in table 5, all subinoculations from Briarcliff (five attempts) and Mme. Butterfly (seven attempts) showing the characteristic local cane lesions of streak have failed to recover the virus. It is assumed that a similar result would be obtained from Columbia, Ophelia, Radiance, Rapture, and Templar since these varieties are uniform with the two tested in response to this virus. Two series of subinoculations were attempted with a view to determining how far the streak virus advanced beyond its recognizable symptoms. In one series buds were taken from Mme. Butterfly 1½ and 4 inches above and ½, 8, and 15 inches below a streak-affected Silver Moon bud 3 months after the latter was set and at a time when the necrotic lesion was still enlarging about the Silver Moon bud. None of these buds conveyed streak to healthy Mme. Butterfly into which they were set. The failure to recover streak from buds ½ inch below and 1½ inches above an active primary necrotic cane lesion indicates that the streak virus is closely confined to the area of visible symptoms in varieties showing this response.

The inserted streak-affected bud is killed by the girdling lesion and the cane above this bud dies gradually, the leaves withering in place and persisting strongly (fig. 7, *B*). The local necrotic lesion may be pruned out, and thereafter the remaining parts of the plant appear free from the disease. Mme. Butterfly has been held for 4 years after the appearance of such lesions, showing no further symptoms. The disease can be induced in such plants in typical form upon reinoculation. Such reinoculation has been uniformly successful in eight attempts in Briarcliff, six in Mme. Butterfly, and one in Ophelia. The second inoculations followed the first after 9 to 18 months, when the first symptoms were apparently inactive. In every instance the typical necrotic effects developed after the second inoculation with no recognizable departure from the effects of the first inoculation. Failure to protect against primary lesions upon reinoculation is usually regarded as evidence that the virus is localized rather than systemic. Such evidence supports the writers' conclusions, from subinoculations and from successful pruning out of streak lesions, that the necrotic effects of streak in hybrid teas such as these are primary local lesions.

A third group of varieties, long considered immune, includes C. F. Meyer, Duchess of Wellington, Joanna Hill, Kaiserin Auguste Viktoria, Margaret McGredy, Ragged Robin, *Rosa hugonis*, *R. nutkana*, Souvenir de Claudius Pernet, and Ulrich Brunner. These varieties developed neither primary necrosis nor systemic symptoms when inoculated with buds of systemically affected varieties or when budded into canes of streak-affected Odorata in routine trials. Several attempts to recover streak by subinoculation failed. However, after long union with streak-affected scions of Silver Moon this apparent immunity broke down in Duchess of Wellington, Kaiserin Auguste Viktoria, and *R. nutkana*, and these varieties expressed systemic symptoms. It appears that the incubation period may be greatly prolonged in some varieties, and also that passage of the streak virus upward across

a union may be far slower than the downward movement. It is therefore necessary to examine the evidence for resistance or immunity in these varieties in further detail.

Two large plants of Odorata naturally infected with streak were budded at various times with C. F. Meyer, Duchess of Wellington, Margaret McGredy, Mme. Butterfly, *Rosa hugonis*, Silver Moon, Souvenir de Claudius Pernet, and Talisman. The Odorata plants showed cane symptoms and also brown leaf markings and senescence patterns of systemic infection at various times during this test. Mme. Butterfly shoots died 4 months after the bud was set, with characteristic necrotic streak symptoms. A Talisman bud developed into a scion branch 8 inches long in 2 months, remained apparently healthy for 4 months, but died with necrotic streak symptoms during the sixth month of contact. Two Silver Moon buds, set one in each plant of Odorata, developed into scion branches about 3 feet long before systemic symptoms appeared in the fourth month. Thus the streak virus is shown to have passed upward, inducing typical necrotic and typical systemic effects in appropriate varieties in 4 to 6 months after budding into streak-affected Odorata. *Rosa hugonis* grew on the streak-affected Odorata for 13 months without showing symptoms, and Margaret McGredy grew for 23 months, developing a strong well-branched shoot 3 feet tall; but no symptoms were expressed, and a subinoculation to Mme. Butterfly at the end of the test was negative. Souvenir de Claudius Pernet grew normally on streak Odorata for 21 months, and subinoculations to Briarcliff and Mme. Butterfly after 8 months were negative.

C. F. Meyer was set in streak Odorata on June 7, 1934, and Duchess of Wellington on November 8, 1934. Both developed vigorous scion branches which remained symptomless until the test was concluded September 28, 1937. On November 1, 1935, healthy Mme. Butterfly was budded into these scions, providing a susceptible test variety separated from the systemically affected Odorata by intermediate canes of C. F. Meyer 30 inches long and Duchess of Wellington 17 inches long. Mme. Butterfly grew vigorously, flowered freely, and ripened fruits, without showing streak effects in 23 months, when thus separated from the streak source by intermediate scions. Mme. Butterfly budded at the same date into Silver Moon, an intermediate susceptible scion on the same Odorata stock, was killed during the third month by the usual black necrotic effects of streak in this variety. This experiment seemed to provide strong evidence that Duchess of Wellington and C. F. Meyer are immune to streak. No further tests were made with the latter variety, but in a parallel trial streak-affected Silver Moon set in a cane of Duchess of Wellington induced typical systemic streak in Duchess of Wellington in 14 months. During this time the Silver Moon scion attained a length of several feet and expressed strong streak symptoms. Subinoculation from Duchess of Wellington to Mme. Butterfly was positive. The correct interpretation of this evidence would appear to be that Duchess of Wellington is susceptible but offers some resistance to streak invasion, particularly to the movement of the streak virus upward across a union.

Like Duchess of Wellington, Kaiserin Auguste Viktoria and *Rosa nutkana* developed systemic streak symptoms after long delay when inoculated with buds of streak-affected Silver Moon. Both these

varieties showed streak 15 months after budding. The inoculated plant of *R. nutkana* carried a healthy scion shoot of Mme. Butterfly on a separate cane from that budded to streak. Necrotic spotting and gradual dieback of Mme. Butterfly became evident 12 months after the Silver Moon bud was inserted; i. e., evidence of systemic spread of the virus was available 3 months before *R. nutkana* expressed symptoms. The well-established Mme. Butterfly scion, 18 months old when the inoculation was made, was killed completely by streak necrosis, and some dieback effects appeared in *R. nutkana* also. Streak infections have been demonstrated by subinoculation before symptoms were evident in other systemically susceptible roses, e. g., Multiflora and *R. wichuraiana*, in routine trials.

Joanna Hill was twice budded with streak from Silver Moon without expressing symptoms. One inoculated plant was held in the greenhouse for 18 months; the other was grown for a year in the field after 4 months' contact with streak in the greenhouse. Ragged Robin was similarly budded twice, one plant was held under greenhouse conditions for 12 months and the other was moved to the field after 3 months' incubation in the greenhouse. No evidence of streak appeared in Ragged Robin. *Rosa hugonis* carried a strong streak-affected Silver Moon scion shoot for 41 months without showing streak, and subinoculation from *R. hugonis* to Mme. Butterfly after 34 months was negative. Ulrich Brunner carried a vigorous streak-affected Silver Moon scion shoot for 24 months without developing streak symptoms and a Mme. Butterfly scion remained healthy on a separate cane. Five plants of Souvenir de Claudius Pernet were inoculated with streak from Silver Moon and were held for 5 months (1 plant), 7 months (2 plants), 11 months (1 plant,) and 22 months (1 plant) without developing streak symptoms.

The data here detailed require the transfer of Duchess of Wellington, Kaiserin Auguste Viktoria, and *Rosa nutkana* to the group of roses subject to systemic invasion by streak. There is further serious doubt as to whether true immunity to streak occurs, since severe and prolonged testing would be required to establish this point. Nevertheless there is good reason to expect that these varieties will prove at least resistant to streak if they chance to be budded or grafted on streak-affected understocks in the usual commercial practice.

Considerable evidence is available that mosaic and streak move slowly through old canes, and this fact would account for the long incubation periods in the varieties that grew sluggishly after budding. A peculiar instance of failure of the streak virus to move upward from roots may be of interest. Healthy scions of Silver Moon were grafted on pieces of roots of streak-affected Odorata, by the method known as piece-root grafting, on March 6, 1935. The object was to demonstrate the occurrence of streak virus in roots of roses. Two scions developed into vigorous plants but showed no symptoms of streak during 13 months in the greenhouse. They were then transplanted to the field and grew an additional 20 months without evidence of streak infection. On December 10, 1937, 33 months after the root grafts were made, the two plants were dug and sample buds and pieces of root handled as buds were set separately in four healthy Mme. Butterfly plants. No streak developed about the buds, but typical streak lesions developed in each Mme. Butterfly plant "budded" with pieces of root. Symptoms were evident in the

fourth month and girdling was complete in the eighth month after budding. Thus streak was recovered from the roots of each plant but from the canes of neither. The presence of the streak virus in rose roots was thus demonstrated by a method more devious than was originally intended. It was not expected that for a period of 33 months the virus would fail to move upward from the roots into shoots of the Silver Moon variety, which is known for tremendous vigor.

The writers have stressed the evaluation of symptoms and the prevalence of these diseases in roses. It has not been practicable to attempt an investigation of transmission of the viruses to plants in other genera of the Rosaceae. It is not unlikely, especially in the light of Thomas' (*16*) work, that other members of this family may be susceptible to one or more of these viruses.

VIRUS INTERACTIONS

The evidence at hand is inadequate to determine whether the viruses discussed in this paper are separate entities or strains of a general rose mosaic group. Typical rose mosaic and the yellow variants agree rather closely in general symptom types produced, in incubation period, in failure of juice inoculations to transmit, and in known host ranges. The streak disease, on the other hand, differs sharply from the mosaic diseases in symptoms and in varietal responses, but is similar with respect to incubation period and modes of transmission so far as is known.

The results of a number of double inoculations may be discussed at this point since the interaction of viruses has been widely used in interpreting relationships. On October 1, 1937, buds of Talisman carrying yellow mosaic of the Talisman strain were set in five Mme. Butterfly plants that had carried typical rose mosaic for a year or more. During the following 10 months the yellow strain was observed to dominate the green type in two plants, but symptoms of the green type were not entirely suppressed. In the other three plants the green pattern continued unchanged with no effect from introduction of the second virus. The Margaret McGredy strain of yellow mosaic introduced into two mosaic-affected Mme. Butterfly plants under like conditions produced no effect on the symptoms expressed during the succeeding 10 months. Yellow mosaic from Margaret McGredy was successfully superimposed on yellow mosaic of the Irish Charm strain in the former variety in a single trial. The second inoculation followed the first by 28 months, and the more brilliant pattern of the second virus was recognizable 4 months later. A single attempt to superimpose the Talisman strain on the Irish Charm strain in the Talisman variety evidently failed. The second virus was inoculated 28 months after the first; no change in response was noted 8 months later.

Typical rose mosaic in Mme. Butterfly, yellow mosaic in Talisman, and healthy Silver Moon and Kaiserin Auguste Viktoria were budded into each of two large Odorata plants in pots on November 1, 1935. Suckers of Odorata were permitted to develop to express the symptom reaction of this stock, and the five-variety system was observed at intervals for nearly 30 months. There was no clear evidence of dominance of one virus over the other. Mme. Butterfly, Odorata, and

Silver Moon expressed symptoms of each virus in each of the two plants; Talisman and Kaiserin Auguste Viktoria showed only yellow mosaic symptoms in one plant and no symptoms in the other. Both mosaic and yellow mosaic expressed prominent symptoms at intervals, one seeming to dominate in one variety, the other in another. At the conclusion of the test both viruses were evidently present in Mme. Butterfly, Odorata, and Silver Moon, in some instances symptoms of both appearing in the same leaf. The logical interpretation would be that the two can exist and multiply together without marked effect on each other. Since both are systemic, this evidence has no important bearing on their relationship, but if it has weight at all, it is against their being strains of a single virus.

Yellow patterns suggesting a mutation from the green to a yellow type were observed in two Mme. Butterfly plants affected with typical rose mosaic, one in the field and one in the greenhouse. Subinoculation from each to healthy Mme. Butterfly reproduced only green mosaic. The possible origin of yellow types from the typical rose mosaic by mutation was therefore not confirmed.

More significance is attached to the production of the primary cane lesions of rose streak in varieties systemically affected with typical rose mosaic. The varieties Briarcliff and Templar each show systemic symptoms of rose mosaic, and each is subject to primary necrosis about the streak-affected bud when the latter disease is introduced. Streak was produced in Briarcliff twice when introduced 4 months after, and 5 months after, mosaic inoculation. Streak symptoms appeared in 27 days in typical form in each plant and remained localized. Mosaic symptoms were unaffected by introduction of the second virus during 14 months' subsequent observation. Similarly, streak was superimposed on mosaic in a Templar plant of long-standing mosaic history. Typical streak developed in 10 weeks, ran its course in 6 months, and was pruned out, leaving typical mosaic effects still evident. The fact that Briarcliff and Templar, systemically infected with the rose mosaic virus, expressed the characteristic primary lesions of streak on subsequent budding is an indication that the streak virus is not closely allied to that of rose mosaic.

SPREAD OF ROSE MOSAIC IN NURSERY PRACTICE

Observers agreed soon after rose mosaic was first recognized that no natural spread occurred under glass. The suspicion that natural spread might occur in the field has been more persistent, but so far as the writers are aware, nearly all field evidence of spread is based on the appearance of crinkle and speckle types herein shown to be unrelated to rose mosaic. The appearance of mosaic in new varieties of roses of comparatively recent seed origin and the occasional presence of high percentages of the disease in certain nurseries would suggest natural spread of authentic rose mosaic. The writers' test plantings in Oregon, New York, Virginia, and Maryland have failed to yield any evidence of natural spread, and all attempts to transmit the disease by means of insects have failed. In the writers' opinion all available evidence for spread of rose mosaic can be accounted for by propagation practices.

No evidence was furnished by the trials described above that rose mosaic spreads in the field in commercial rose nurseries in Oregon or

in a general farming area at Babylon, N. Y. Nor was there evidence of spread in 1933 at Arlington, Va., when 15 healthy Mme. Butterfly plants were grown near mosaic sources throughout the season and then returned to the greenhouse for observation. Again in 1934, 52 healthy Briarcliff, 31 Mme. Butterfly, 3 Ophelia, 15 Rapture, and 2 Canina plants showed no spread of mosaic from adjacent proved source plants at the Arlington farm. At Beltsville, Md., 34 plants of Radiance, 5 plants each of Briarcliff, Canina, Joanna Hill, Mme. Butterfly, Manetti, Multiflora, Odorata, and Texas Wax, 1 plant of Ragged Robin, and 2 of *Rosa hugonis*, grown adjacent to proved mosaic, yellow mosaic, and streak source plants from March or April 1935 through September 1936, showed no spread of any of these three diseases. In view of the fact that several of the varieties included in these trials are known to express mosaic symptoms with a high degree of reliability, these results offer strong evidence that spread of mosaic does not commonly occur in these localities. A separate test for natural spread of rose streak at Beltsville was also negative. No spread of the disease from affected plants to 80 plants of healthy Silver Moon, a readily affected variety, could be detected at any time during a 4-year exposure.

It is well known that mosaic is carried in cuttings or scions taken from affected plants. However, field-budding practices as a means of pseudo-natural spread seem to have been overlooked. Two general practices for maintaining a supply of Manetti wood for cuttings are followed in the West. In the first, a "mother block" is maintained as a source of cuttings, and hundreds of cuttings are rooted from each established plant each year that it is in use. Thus scions are never worked on the plants in the mother block that provides the cuttings.

In the second procedure, the mother block is eliminated and cuttings are collected at random from the tops of field-budded plants when the latter are cut back to force the inserted buds. Such cuttings from budded plants are subject to the hazard of contact with buds that may have carried mosaic. In each practice the Manetti cuttings are lined out in the field for rooting, and are budded the following summer. Should a mother-block plant become infected, all its vegetative progeny would carry the mosaic virus and transmit it to previously healthy buds worked on them. However, if the mother block is free from mosaic, the disease will appear only in plants grown from infected budwood. When cuttings are taken from budded plants, any cutting from a diseased plant may transmit the disease to healthy inserted buds in the next season. Mosaic bud stock thus infects the healthy bud stock of the following season. Such infection is avoided by the mother-block system. The practice of taking cuttings for understocks from the tops of budded plants is doubtless a sound commercial practice from other viewpoints, but it provides an efficient means of preservation and dissemination of virus diseases such as rose mosaic, yellow mosaics, and, in varieties subject to systemic invasion, rose streak. As far as the writers have been able to determine, the higher percentages of mosaic recorded in table 4 are from nurseries that root cuttings from budded plants. In view of the difficulty of recognizing mosaic in Manetti and Ragged Robin stocks as well as in a large number of budded varieties, it is manifestly impossible to recognize the presence of rose mosaic in affected understocks propagated from budded plants. A high percentage of infection can thus be built up without conspicu-

ous symptom expression. When a variety capable of expressing strong mosaic symptoms is worked into such understocks, a sudden appearance of mosaic may result, and will usually be attributed to natural spread. The practice of taking cuttings from budded stocks is so well suited to preservation of the virus diseases and to their dissemination to new varieties that it seems adequate to account for all observed spread of rose mosaic.

CONTROL

The control measures outlined below are those indicated by the writers' evidence on the nature and behavior of the diseases here discussed. Tests of these measures on a nursery scale have not been made.

Practical control of mosaic in propagation on Manetti understocks can be accomplished by establishing a disease-free mother block. This is desirable since mosaic in Manetti stocks (and others) is difficult to diagnose in the field. A small block of plants can be checked by budding to varieties readily diagnosed when affected, such as Mme. Butterfly, Ophelia, or Rapture, and plants thus proved healthy can thereafter be reserved as healthy mother plants. The test variety may be budded into the canes of the mother block in the field, or buds from the mother block may be set in canes of the test varieties. If both stock and scion grow vigorously, a period of 3 or 4 months is adequate for a test. A mosaic-free mother block may be expanded and renewed as desired by means of cuttings. With such a proved source of healthy cuttings available, mosaic will occur only as a result of using infected buds, and will not spread to other varieties.

Selection of budwood from healthy plants is also important. This is easily feasible in the varieties that are severely injured when affected. In those varieties in which mosaic symptoms are more difficult to detect, affected buds sometimes may be selected even by competent propagators. Little harm will result from this, however, provided the mother-block system is used. The use of this system should be feasible for other stocks as well as Manetti.

The procedure outlined for control of mosaic is suggested for the control of the yellow mosaics and of streak. The varieties Mme. Butterfly, Ophelia, or Rapture express yellow mosaics clearly; buds of these varieties die when set in streak-affected stocks.

The possibility remains that some of these diseases may be spread by insect vectors in some localities or in some seasons, but there is no evidence of such spread at present. Control recommendations are therefore confined to elimination of known modes of propagation of these diseases.

DISCUSSION

Slow movement of the mosaic and streak viruses in inoculated plants and slow expression of symptoms are not believed to weaken the validity of the writers' index trials, since responsive test varieties were used and since tests were not considered conclusive unless scion and stock grew actively. If others using the index method will observe the same precautions, conclusive readings may be expected within 3 or 4 months. Prolonged delay in the expression of symptoms of both mosaic and streak was observed in varieties that express symptoms sporadically after infection is fully systemic. In such varieties the

physiology of symptom expression is involved as well as the physiology of virus movement. Where movement of the virus has been demonstrated to be slow by subinoculation trials, it seems explainable according to established principles laid down by Bennett *(2)* and others.

It has been suggested above that White (*23*) apparently had a yellow mosaic, and possibly streak, in his collections. The rose virus disease described by Valleau (*18*) and by Johnson and Valleau (*9*) is apparently typical rose mosaic in a variety that produces only the watermark-ring series of symptoms. The last-named authors have suggested that their collection is a specimen of White's rose mosaic. Milbrath's (*12*) albication seems to agree in symptoms with one of the writers' yellow mosaic collections, although nontransmissible albication patterns occur in roses. Thomas (*16*) illustrates a pattern closely similar to typical rose mosaic induced in the Belle of Portugal variety by transfer from mosaic-affected apple. Christoff (*6*) mentions a mosaic of *Rosa gallica* L. transmissible to apple and pear. Unfortunately the writers do not have symptoms of their viruses in these roses for comparison.

The streak disease, which produces necrotic effects on some rose varieties, appears to differ from wilt and dieback, described by Grieve (*8*) from Australia, and from Gigante's (*7*) new virus of rose in Italy, both in symptoms produced and in modes of transmission. Both Grieve and Gigante stress symptoms in young leaves, whereas streak symptoms are recognizable only as the leaves approach full size and after. Moreover, both the Australian and the Italian viruses are juice-transmissible, and the former is filterable. Gigante considers his disease distinct from Grieve's and from rose mosaic.

SUMMARY

The symptoms of rose mosaic in Mme. Butterfly are prominent chlorotic areas feathering away from the midribs of leaflets, and also ring, oak-leaf, and watermark patterns. Various combinations of these symptoms, with various degrees of intensity, are expressed in other rose varieties and species. Some rose varieties and the understocks Manetti, Ragged Robin, and Texas Wax express symptoms too irregularly for accurate direct diagnosis.

Yellow mosaics are characterized by brighter and lighter yellow patterns than are found in typical rose mosaic.

The streak disease of roses produces brown rings, brown or yellowish vein banding, and senescence patterns in leaves, and brownish or greenish ring markings in canes. Certain hybrid tea roses are subject to necrotic primary lesions in canes.

Many chlorotic speckle and crinkle patterns, including the disorder in Manetti known as rattlesnake in the Northwest, are unrelated to rose mosaic, and have not been shown to be of virus etiology.

Field diagnosis of mosaic and streak is unreliable because of the tendency of the symptoms to become masked in some varieties.

The occurrence of rose mosaic has been confirmed by the transfer from 17 varieties of roses from 7 States and the Province of Ontario, but the disease has been found to be prevalent only in association with certain propagation practices. Index tests of understocks from Oregon, California, Texas, and elsewhere show that the disease is less prevalent than was formerly claimed.

Yellow mosaics have been found in scattered single plants in four localities.

Streak is known from Maryland, New York, Texas, Virginia, and the District of Columbia. This disease affected 30 percent of the roses in one collection of varieties.

Mosaic, yellow mosaics, and streak have been transmitted only by tissue union, i. e., by various styles of budding or grafting. No transmission resulted when buds were removed before union occurred. The minimum incubation periods recorded for the three virus types are all of the order of 20 days. In less responsive varieties and under less favorable conditions incubation may require several months.

No seed transmission of mosaic or streak was evident in small populations of seedlings from affected plants.

No evidence of insect transmission was obtained from trials of 31 species of insects for mosaic nor from tests of 34 insect species for streak.

Strawberry crinkle was not transmitted to rose by *Philaenus leucophthalmus* (L.) nor by *Capitophorus fragaefolii* (Ckll.). Red raspberry mosaic was not transmitted from red raspberry to rose by *Amphorophora rubi* (Kalt.), nor aster yellows from China aster to rose by *Macrosteles divisus* (Uhler).

All roses adequately tested have proved susceptible to rose mosaic and yellow mosaics, but differences are found in intensity of symptom expression, i. e., in degree of tolerance. Rose varieties and species fall into three groups with respect to streak reactions: (1) Those susceptible to systemic infection, (2) those subject to primary necrotic lesions in canes, and (3) those apparently resistant or immune to infection.

No natural spread of mosaic or streak has been detected in field trials in Maryland, New York, Oregon, and Virginia. It is indicated that the nursery practice of propagating roses on rooted cuttings taken from the tops of budded understocks of the previous season is the probable mechanism of the apparent spread of rose mosaic in nature.

It is suggested that rose mosaic, and probably also yellow mosaics and streak, may be controlled by using understocks derived from a mother block indexed to assure freedom from these diseases.

The available evidence is considered inadequate for determining whether rose mosaic and five yellow mosaics are closely allied. The streak virus is believed to be unrelated to the mosaics and to be unlike the necrotic diseases of rose described by Grieve from Australia and by Gigante from Italy.

LITERATURE CITED

(1) ANONYMOUS.
1930. INFECTIOUS CHLOROSIS OF THE ROSE. A SUMMARY OF PRESENT KNOWLEDGE CONCERNING AN IMPORTANT PLANT MALADY. Florists' Exch. 73 (4): 46, illus.

(2) BENNETT, C. W.
1937. CORRELATION BETWEEN MOVEMENT OF THE CURLY TOP VIRUS AND TRANSLOCATION OF FOOD IN TOBACCO AND SUGAR BEET. Jour. Agr. Res. 54: 479–502, illus.

(3) BRIERLEY, PHILIP.
1935. STREAK, A VIRUS DISEASE OF ROSES. (Abstract.) Phytopathology 25: 7–8.

(4) ———
1935. SYMPTOMS OF ROSE MOSAIC. (Abstract.) Phytopathology 25: 8.

(5) Brierley, Philip, and Smith, Floyd F.
1940. spread of rose virus diseases . . . Amer. Nurseryman 72 (1): 5–8, illus.
(6) Christoff, Alexander.
1935. mosaikfleckigkeit, chlorose und stippenfleckigkeit bei äpfeln, birnen und quitten. Phytopath. Ztschr. 8: [285]–296, illus.
(7) Gigante, R.
1936. una nuova virosi della rosa in italia. [Roma] R. Staz. di Patol. Veg. Bol. (n. s.) 16: 76–94, illus.
(8) Grieve, B. J.
1931. "rose wilt" and "dieback." a virus disease of roses occurring in australia. Austral. Jour. Expt. Biol. and Med. Sci. 8: [107]–121, illus.
(9) Johnson, E. M., and Valleau, W. D.
1935. the ring symptom of virus diseases of plants. Ky. Agr. Expt. Sta. Bul. 361: [239]–263, illus.
(10) Maerz, A., and Paul, M. Rea.
1930. a dictionary of color. 207 pp., illus. New York [etc.].
(11) Milbraith [Milbrath], D. G.
1930. infectious chlorosis of the rose . . . West. Florist 13 (29): 29–30.
(12) Milbrath, D. G.
1930. a discussion of the reported infectious chlorosis of the rose. Calif. Dept. Agr., Monthly Bul. 19: [535]–544, illus. (*Also in* Florists' Exch. 74 (13): 11, 33.)
(13) Nelson, Ray.
1930. infectious chlorosis of the rose. (Abstract.) Phytopathology 20: 130.
(14) Ridgway, Robert.
1912. color standards and color nomenclature. 43 pp., illus. Washington, D. C.
(15) Smith, Floyd F.
1940. certain sucking insects causing injury to rose. Jour. Econ. Ent. 33: 658–662, illus.
(16) Thomas, H. Earl.
1937. apple mosaic. Hilgardia 10: 581–588, illus.
(17) ——— and Massey, L. M.
1939. mosaic diseases of the rose in california. Hilgardia 12: 647–663, illus.
(18) Valleau, W. D.
1932. a virus disease of plum and peach. Ky. Agr. Expt. Sta. Bul. 327: 89–103, illus.
(19) White, R[ichard] P.
1928. an infectious chlorosis of rose. N. J. Agr. Expt. Sta. Ann. Rpt. (1927–28) 49: 278, illus.
(20) ———
1929. diseases of ornamental plants. N. J. Agr. Expt. Sta. Ann. Rpt. (1928–29) 42: 42–43.
(21) ———
1930. an infectious chlorosis of rose. (Abstract.) Phytopathology 20: 130.
(22) ———
1930. an infectious chlorosis of rose. Amer. Rose Ann. 15: 87–90, illus.
(23) ———
1932. chloroses of the rose. Phytopathology 22: 53–69, illus.
(24) ———
1934. the effect of mosaic on bloom production of the talisman rose. Phytopathology 24: 1124–1125.
(25) Yerkes, G. E., Scott, L. B., and Swingle, C. F.
1933. manetti rose stocks should be mature before digging. Florists' Exch. 81 (35): 19, 21, illus.

EFFECT OF DAY LENGTH AND TEMPERATURE ON THE FLOWERING AND GROWTH OF FOUR SPECIES OF GRASSES[1]

By H. M. BENEDICT

Associate physiologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In attempts to carry on physiological investigations with native grasses in the greenhouse during the winter months, the writer has often encountered difficulty in obtaining flower production and good vegetative growth. Some of the grasses when grown under winter greenhouse conditions produce a low tufted top growth and never send up flower stalks. Other species produce only a few leaves and then seem to stop growing until late in the winter or early spring. As a result of the work of Garner and Allard (*4*, *5*, *6*),[2] Tincker (*9*, *10*), Adams (*1*, *2*, *3*), Gilbert (*7*), Thompson (*8*), and others, it was thought that lengthening the day or varying the temperature might bring about increased growth and production of flower stalks in those species that usually do not grow well in the greenhouse during the winter.

In this paper are presented the results of experiments on the effects of different day lengths and temperatures on bluestem (*Agropyron smithii* Rydb.), locally known as western wheatgrass, blue grama (*Bouteloua gracilis* (H. B. K.) Lag.), bluejoint turkeyfoot (*Andropogon furcatus* Muhl.), locally known as big bluestem, and switchgrass (*Panicum virgatum* L.). The first two species are the most common grasses in the short-grass plains and make fairly good growth but rarely flower in the greenhouse during the winter season. The last two species are common in the tall-grass prairies and, at least at Cheyenne, Wyo., make very little growth under winter greenhouse conditions.

METHODS

Three series of experiments were carried out, the first and third during the winter when the days are naturally short and greenhouse temperatures can be kept low, and the second in the summer when the days are naturally long and greenhouse temperatures become high during the day. In the first series plants of *Agropyron smithii* and *Bouteloua gracilis* were grown in seed flats 16 by 24 by 4 inches, 100 plants to a flat, from October 21, 1934, to March 21, 1935. The plants in this series (series 1) were grown in 8-hour, 20-hour, and natural day lengths at a temperature of 60° F. and in a natural day length at a temperature of 75°. In the second series, plants of the same two species

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[2] Italic numbers in parentheses refer to Literature Cited, p. 670.

(5) BRIERLEY, PHILIP. and SMITH, FLOYD F.
1940. SPREAD OF ROSE VIRUS DISEASES . . . Amer. Nurseryman 72 (1): 5–8, illus.
(6) CHRISTOFF, ALEXANDER.
1935. MOSAIKFLECKIGKEIT, CHLOROSE UND STIPPENFLECKIGKEIT BEI ÄPFELN, BIRNEN UND QUITTEN. Phytopath. Ztschr. 8: [285]–296, illus.
(7) GIGANTE, R.
1936. UNA NUOVA VIROSI DELLA ROSA IN ITALIA. [Roma] R. Staz. di Patol. Veg. Bol. (n. s.) 16: 76–94, illus.
(8) GRIEVE, B. J.
1931. "ROSE WILT" AND "DIEBACK." A VIRUS DISEASE OF ROSES OCCURRING IN AUSTRALIA. Austral. Jour. Expt. Biol. and Med. Sci. 8: [107]–121, illus.
(9) JOHNSON, E. M., and VALLEAU, W. D.
1935. THE RING SYMPTOM OF VIRUS DISEASES OF PLANTS. Ky. Agr. Expt. Sta. Bul. 361: [239]–263, illus.
(10) MAERZ, A., and PAUL, M. REA.
1930. A DICTIONARY OF COLOR. 207 pp., illus. New York [etc.].
(11) MILBRAITH [MILBRATH], D. G.
1930. INFECTIOUS CHLOROSIS OF THE ROSE . . . West. Florist 13 (29): 29–30.
(12) MILBRATH, D. G.
1930. A DISCUSSION OF THE REPORTED INFECTIOUS CHLOROSIS OF THE ROSE. Calif. Dept. Agr., Monthly Bul. 19: [535]–544, illus. (*Also in* Florists' Exch. 74 (13): 11, 33.)
(13) NELSON, RAY.
1930. INFECTIOUS CHLOROSIS OF THE ROSE. (Abstract.) Phytopathology 20: 130.
(14) RIDGWAY, ROBERT.
1912. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C.
(15) SMITH, FLOYD F.
1940. CERTAIN SUCKING INSECTS CAUSING INJURY TO ROSE. Jour. Econ. Ent. 33: 658–662, illus.
(16) THOMAS, H. EARL.
1937. APPLE MOSAIC. Hilgardia 10: 581–588, illus.
(17) ——— and MASSEY, L. M.
1939. MOSAIC DISEASES OF THE ROSE IN CALIFORNIA. Hilgardia 12: 647–663, illus.
(18) VALLEAU, W. D.
1932. A VIRUS DISEASE OF PLUM AND PEACH. Ky. Agr. Expt. Sta. Bul. 327: 89–103, illus.
(19) WHITE, R[ICHARD] P.
1928. AN INFECTIOUS CHLOROSIS OF ROSE. N. J. Agr. Expt. Sta. Ann. Rpt. (1927–28) 49: 278, illus.
(20) ———
1929. DISEASES OF ORNAMENTAL PLANTS. N. J. Agr. Expt. Sta. Ann. Rpt. (1928–29) 42: 42–43.
(21) ———
1930. AN INFECTIOUS CHLOROSIS OF ROSE. (Abstract.) Phytopathology 20: 130.
(22) ———
1930. AN INFECTIOUS CHLOROSIS OF ROSE. Amer. Rose Ann. 15: 87–90, illus.
(23) ———
1932. CHLOROSES OF THE ROSE. Phytopathology 22: 53–69, illus.
(24) ———
1934. THE EFFECT OF MOSAIC ON BLOOM PRODUCTION OF THE TALISMAN ROSE. Phytopathology 24: 1124–1125.
(25) YERKES, G. E., SCOTT, L. B., and SWINGLE, C. F.
1933. MANETTI ROSE STOCKS SHOULD BE MATURE BEFORE DIGGING. Florists' Exch. 81 (35): 19, 21, illus.

EFFECT OF DAY LENGTH AND TEMPERATURE ON THE FLOWERING AND GROWTH OF FOUR SPECIES OF GRASSES[1]

By H. M. BENEDICT

Associate physiologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

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METHODS

Three series of experiments were carried out, the first and third during the winter when the days are naturally short and greenhouse temperatures can be kept low, and the second in the summer when the days are naturally long and greenhouse temperatures become high during the day. In the first series plants of *Agropyron smithii* and *Bouteloua gracilis* were grown in seed flats 16 by 24 by 4 inches, 100 plants to a flat, from October 21, 1934, to March 21, 1935. The plants in this series (series 1) were grown in 8-hour, 20-hour, and natural day lengths at a temperature of 60° F. and in a natural day length at a temperature of 75°. In the second series, plants of the same two species

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were grown in seed flats, 77 plants to a flat, in an 8-hour and a 20-hour day, from June 11, 1935, until October 4, 1935. During this period the temperature was maintained as close to 80° as possible, although during the middle of the day it was often several degrees higher. The third series contained plants of *Agropyron smithii*, *Andropogon furcatus*, *Bouteloua gracilis*, and *Panicum virgatum*. These plants were grown in seed flats from November 20, 1936, to March 24, 1937. The plants were all thinned to 50 per flat and grown under the following six conditions, with duplicate flats of each species placed in each treatment.

(1) In a natural, or short, day length at a day and night temperature of 75° F.
(2) In a natural day length at a day temperature of 75° F. and a night temperature of 60°.
(3) In an 18-hour, or long, day length at a day and night temperature of 75° F.
(4) In an 18-hour day at a temperature of 75° F. during the day and a night temperature of 60°.
(5) In a natural day length until January 20 and then in an 18-hour day length until March 24, at a day and night temperature of 75° F.
(6) In an 18-hour day length until January 20 and then in a natural day length until March 24 at a day and night temperature of 75° F.

The desired temperatures in the three series of experiments were maintained by hand operation of the greenhouse ventilators. This method admittedly allows for some variation, but except for the summer series this was never more than 5° F., according to the thermograph records that were kept. The night temperature of 60° used in series 3 was obtained by removing the plants from the greenhouse to a propagating house for the required length of time.

The 18- and 20-hour day lengths were obtained by burning incandescent lamps from shortly before sundown for as many hours as were necessary to give the required day length. The intensity of the radiation of the incandescent lamps at the surface of the soil was 35 foot-candles. The areas lighted by the lamps were screened so as to prevent any stray radiations from reaching areas that were to be kept dark. The 8-hour day length was maintained by covering the plants with a lightproof canvas from 4 p. m. until 8 a. m. daily. This canvas was suspended on a frame 54 inches high, closed at either end by wallboarding that could be removed during the day. It was not possible to observe any light in the compartment thus formed. The temperature inside never exceeded that of the rest of the greenhouse by more than 2° F.

By thoroughly mixing the soil before placing it in the flats in all three experiments, care was taken to insure that no differences in the soil existed between treatments.

According to information supplied by the United States Weather Bureau, the average length of daily sunlight during the time the plants were being grown in the winter was about 10¼ hours. Thus the natural day length in the winter will be considered a short day.

The dry weight per plant was used as the criterion of growth of the plants subjected to the various treatments. The dry weights were determined by carefully washing the soil from the plants, separating the roots from the tops, placing both in an oven at 100° C. for an hour, and then drying them to constant weight at 80°.

EXPERIMENTAL RESULTS AND DISCUSSION

EFFECT OF DAY LENGTH AND TEMPERATURE ON FLOWERING

The number of days elapsing between the date of planting and the date on which at least three plants in a given treatment showed flower stalks are given in table 1. The results presented here indicate that *Agropyron smithii* is a long-day plant and that the proper temperature alone will not bring about flowering. This is brought out by the fact that under the conditions of these experiments plants of this species flowered in an 18- or 20-hour day when the temperatures in which they were growing ranged from 60° to 80° F. but failed to bloom when growing at these temperatures in an 8- or 10-hour day. It will be noted that plants of this species in series 3 failed to bloom in an 18-hour day when the night temperature was lowered from 75° to 60°. This failure to flower may have been due entirely to the low temperature, but since the plants of this series that did flower did so on the last day of the experiment it seems possible that the plants were not grown long enough to reach the flowering stage.

TABLE 1.—*Number of days between the date of planting and the appearance of first bloom of different grasses grown in different day lengths and temperatures*

Treatment		Time from planting to blooming in indicated species			
Day length	Temperature conditions	*Agropyron smithii*	*Andropogon furcatus*	*Bouteloua gracilis*	*Panicum virgatum*
	°F.	Days	Days	Days	Days
Series 1 (winter):					
8-hour day	60	[1]		[1]	
Natural, or 10-hour, day	60	[1]		[1]	
20-hour day	60	149		[1]	
Natural, or 10-hour, day	75	[1]		93	
Series 2 (summer):					
8-hour day	80	[1]		72	
20-hour day	80	98		100	
Series 3 (winter):					
Natural, or 10-hour, day	75 throughout	[1]	[1]	70	72
Natural, or 10-hour, day	75 day; 60 night	[1]	101	[1]	93
18-hour day	75 throughout	120	[1]	113	[1]
18-hour day	75 day; 60 night	[1]	[1]	[1]	[1]
10-hour, 2 months; 18-hour, 2 months.	75 throughout	[1]	[1]	70	[1]
18-hour, 2 months; 10-hour, 2 months.	75 throughout	[1]	[1]	87	[1]

[1] Failed to bloom.

As shown in table 1, plants of *Bouteloua gracilis* flowered in an 18- and 20-hour day as well as in an 8- or 10-hour day when the temperature remained at 75° F. or above, but failed to flower in any day length when the temperature dropped to 60° for even a few hours daily. The plants grown in the shorter day lengths flowered earlier than those grown in the longer day lengths. These results indicate that *B. gracilis* is an indeterminate type of plant as far as its blooming response to day length is concerned and that a fairly high temperature is necessary to produce flowering.

Plants of *Andropogon furcatus* bloomed in the 10-hour day at a temperature of 75° F. during the day and 60° during the night, but

did not bloom in this short day when the temperature was kept at 75° throughout, nor did the plants of this species bloom in the 18-hour, or long, day. These results seem to indicate that *A. furcatus* is a short-day plant and that temperature alone does not determine whether it will flower. The plants of this species grown at a temperature of 75° in the short day may not have flowered because of the extremely small growth they made, having a total dry weight of only 23 mg.

Plants of *Panicum virgatum* bloomed when grown in a short day, regardless of the temperature, although lowering the temperature at night retarded the blooming date somewhat. This would indicate that *P. virgatum* is a short-day plant and that the temperatures used in this experiment have little influence in determining whether or not these plants will flower.

In setting up the treatments in which the plants were grown, first in a short day and then in a long day or first in a long day and then in a short day, it was thought that the day length unfavorable to flowering when applied first would cause the plants to vegetate and that then changing to the day length favorable for flowering would result in these plants coming into flower very rapidly. However, it will be noted from table 1 that only the plants of *Bouteloua gracilis* flowered in these treatments and that the plants of this species were able to flower in either a continuous long or a continuous short day. These results seem to show for the other three grasses under the conditions of these experiments that the effect of growing them for 2 months in a day length unfavorable for blooming was not overcome by growing them afterward for 2 months in a day length favorable for blooming. They also show that a day length unfavorable for blooming was able quickly to overcome the effects of growing the plants for 2 months in a day length favorable for blooming. For example, plants of *Panicum virgatum* growing in a long day did not flower while those growing in a short day flowered in 72 days. Yet when plants growing in the short day for 61 days were placed in a long day they did not flower, nor did the plants of this species flower when transferred to a short day after being in a long day for 2 months.

The classification of *Agropyron smithii* as a long-day plant, *Andropogon furcatus* and *Panicum virgatum* as short-day plants, and *Bouteloua gracilis* as a plant of indeterminate day length is borne out by the time of the year in which these plants come into flower when growing under natural conditions. Plants of *A. smithii* bloom in the latter part of June when the days are longest. Plants of *B. gracilis* will send up flower stalks throughout the summer. Plants of *A. furcatus* and *P. virgatum* come into flower around the middle of August, when the days are becoming shorter.

EFFECT OF DAY LENGTH AND TEMPERATURE ON GROWTH

The dry-weight determinations on the plants of the first series were ruined by an overheated oven and so cannot be presented. The dry weights per plant of the roots, tops, and total plant of the species grown in series 2 and 3 are shown in tables 2 and 3, respectively. The values presented in table 3 are the means of the two replications of each species in each treatment. In both series, with one exception, the weights of the individual parts of a plant of one species always

varied in the same direction with change in treatment. Thus, if the roots of the plants of one species increased when the day length was increased, the tops also increased in dry weight and naturally the entire plant showed the same trend. This makes it possible to discuss together instead of separately the results of the treatments of the plants of the different species.

TABLE 2.—*Dry weight per plant of Agropyron smithii and Bouteloua gracilis (series 2) grown in the summer of 1935 under different day lengths at 80° F.*

Species and treatment	Dry weight per plant		
	Root	Top	Total
Agropyron smithii:	*Gram*	*Gram*	*Gram*
8-hour day	0.056	0.131	0.187
20-hour day	.053	.124	.177
Bouteloua gracilis:			
8-hour day	.255	.436	.691
20-hour day	.289	.620	.909

TABLE 3.—*Dry weight per plant of grasses of series 3 when grown in different day lengths and temperatures*

Species and plant part	Dry weight per plant under indicated conditions					
	Normal or 10-hour day		18-hour day		10-hour day, 2 months; 18-hour day, 2 months; 75° F. throughout	18-hour day, 2 months; 10-hour day, 2 months; 75° F. throughout
	75° F. throughout	75° F. day, 60° F. night	75° F. throughout	75° F. day, 60° F. night		
Agropyron smithii:	*Gram*	*Gram*	*Grams*	*Gram*	*Gram*	*Gram*
Roots	0.176	0.235	0.194	0.228	0.236	0.243
Tops	.312	.420	.346	.372	.408	.375
Total	.488	.655	.540	.600	.644	.618
Andropogon furcatus:						
Roots	.009	.023	.311	.324	.030	.113
Tops	.014	.033	.545	.587	.098	.155
Total	.023	.056	.856	.911	.128	.268
Bouteloua gracilis:						
Roots	.184	.091	.315	.206	.235	.323
Tops	.560	.218	.825	.571	.731	.652
Total	.744	.309	1.140	.777	.966	.975
Panicum virgatum:						
Roots	.010	.074	.299	.223	.024	.101
Tops	.006	.053	.721	.504	.052	.063
Total	.016	.127	1.020	.727	.076	.164

The results of the dry-weight determinations (table 2) indicate that day length has little effect on the growth of plants of *Agropyron smithii*, provided the temperature is kept constant. This is brought out by the fact that in series 2 the plants of this species had about the same dry weight when grown in either the long or the short day and that in series 3, when grown at a temperature of 75° F. throughout, the plants in the long day had a slightly greater dry weight than those

in the short day but, when grown at a night temperature of 60°, the plants in the short day had a greater dry weight. The variations obtained between replications in series 3 indicate that not much importance can be attached to the differences noted between the dry weights of the plants growing in the different day lengths at the same temperature.

The results presented in table 3 also indicate that reducing the temperature at night causes an increase in the growth of plants of *Agropyron smithii*, since the plants growing in the low night temperature had the greater dry weight in both day lengths. This increase may be due to the reduced respiration rate occurring at the lower temperature, which would mean that less of the material manufactured during the day would be respired away at night. It can be seen that the plants in the short day showed a greater increase in dry weight when subjected to the lower temperatures than did the plants in the long day. This may be due to the fact that the former were in the low temperature for about 14 hours daily and the latter for only 6 hours daily. No explanation can be offered as to why the plants of *A. smithii* that were grown in a short day and then in a long day or were grown in a long day and then in a short day had somewhat greater dry weights than the plants grown continuously in a long or a short day.

The results of the dry-weight determinations on plants of *Andropogon furcatus* (table 3) require little discussion. They show that increasing the length of day greatly increases the growth or dry weight of the plants of this species, regardless of the temperature used. They also indicate that lowering the night temperature increases the dry weight of the plants, although not to the extent that increasing the day length does. This increase in dry weight in the lower night temperature might possibly be explained on the basis of reduced respiration rate, as in the case of plants of *Agropyron smithii*.

The results of the dry-weight determinations on plants of *Bouteloua gracilis* (tables 2 and 3) show that plants of this species are capable of making fairly good growth in either a long or a short day, but that increasing the day length markedly increases the growth of the plants, provided the temperature remains constant. The fact that the dry weights of the plants grown first in a short day and then in a long day or first in a long day and then in a short day were about intermediate to the dry weights of the plants grown throughout in a long or a short day indicates that a short day length is not so detrimental to the growth of this species as it is to that of *Andropogon furcatus* and *Panicum virgatum*. In the case of these latter species the dry weights of the plants grown in a short day and then in a long day or in a long day and then in a short day were nearer to those of the plants grown entirely in the short day.

It is interesting to note that lowering the night temperature in which the plants of *Bouteloua gracilis* were growing caused a reduction in the dry weight of the plants in both day lengths. It can also be seen that reducing the night temperature reduced by more than half the dry weights of the plants growing in the short day, but reduced by only a third those of the plants growing in the long days. These results would seem to indicate that plants of this species require a higher temperature than those of the other species for their growth

and that lowering the night temperature to 60° F. interferes with their metabolic processes to such an extent that the ensuing reduction in food production cannot be balanced by any reduced respiration rate that may result from the lower temperature. This is further borne out by the fact that the plants growing in the short day, which were subjected to the low temperatures for 14 hours daily, suffered a greater reduction in their dry weight than the plants growing in a long day, which were subjected to the low temperatures for only 6 hours daily. It will be remembered that the plants growing in the low night temperatures were the only ones that did not flower. This would further emphasize the high-temperature requirement of plants of *B. gracilis*.

The results of the dry-weight determinations on the plants of *Panicum virgatum* (table 3) show that the growth of this species is much greater in a long day than in a short day, since the dry weights of the plants under both temperature conditions were much greater in the long day. The results also indicate that lowering the temperature from 75° to 60° F. during the night increases the growth of the plants in a short day but decreases their growth in a long day.

The ratios obtained by dividing the dry weights of the roots by the dry weights of the tops of the plants grown in series 2 and 3 are shown in table 4. These results indicate that day length affects the relative growth of the roots as compared to that of the tops of plants of *Andropogon furcatus* and *Panicum virgatum* but not of *Agropyron smithii* and *Bouteloua gracilis*. Thus the root-top ratios of the former two species were greater in the short days than in the long days under both temperature conditions, but the root-top ratios of the latter two species did not differ significantly in the two day lengths. There is some evidence that changing the plants from a long day to a short day tended to increase the proportion of roots produced more than did changing the plants from a short day to a long day. This is brought out by the fact that the root-top ratios of the plants of all the species worked with were greater when the plants were grown in a long day for 2 months and then in a short day for 2 months than when they were grown in a short day for 2 months and then in a long day for 2 months. However, plants of *A. furcatus* and *P. virgatum* showed much greater differences in root-top ratios between these two treatments than did the plants of *A. smithii* and *B. gracilis*.

TABLE 4.—*Root-top ratio of plants of grasses of series 2 and 3 when grown in different day lengths and temperatures*

Treatment		Root-top ratio of indicated species			
Day length	Temperature conditions	*Agropyron smithii*	*Andropogon furcatus*	*Bouteloua gracilis*	*Panicum virgatum*
Series 2 (summer):	°F.				
8-hour day	80	0.43		0.58	
20-hour day	80	.43		.47	
Series 3 (winter):					
Natural, or 10-hour, day	75 throughout	.56	0.64	.33	1.67
Natural, or 10-hour, day	75 day, 60 night	.56	.70	.42	1.40
18-hour day	75 throughout	.56	.57	.38	.41
18-hour day	75 day, 60 night	.61	.55	.36	.44
10-hour, 2 months; 18-hour, 2 months.	75 throughout	.58	.31	.32	.46
18-hour, 2 months; 10-hour, 2 months.	75 throughout	.65	.73	.50	1.60

The results also indicate that a low night temperature has little or no effect on the relative amounts of roots and shoots produced by the plants, since the root-top ratios of the plants of the four species did not differ appreciably when grown in a high or a low night temperature in the same day length.

The plants of *Agropyron smithii*, *Andropogon furcatus*, *Bouteloua gracilis*, and *Panicum virgatum* after being grown for 11 weeks at

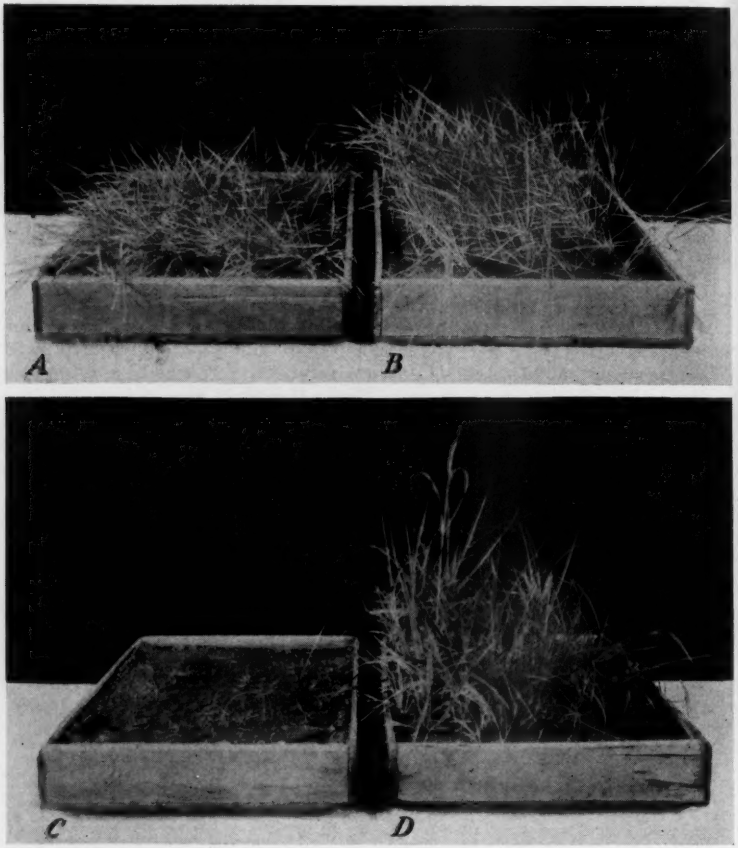

FIGURE 1.—Plants grown for 11 weeks at 75° F.: *Agropyron smithii* grown (*A*) in a 10-hour day and (*B*) in an 18-hour day; *Andropogon furcatus* grown (*C*) in a 10-hour day and (*D*) in an 18-hour day.

75° F. throughout, some in a 10-hour and some in an 18-hour day, are shown in figures 1 and 2. The exceedingly poor growth made by the plants *A. furcatus* and *P. virgatum* in the 10-hour day is brought out in figure 1, *C*, and figure 2, *C*. Figures 1 and 2 also show that a long day increased the height of the plants of all four species under investigation. However, there was no significant difference between the dry weights of the plants of *A. smithii* grown in the two treatments.

CONCLUSIONS

In general, the results presented in this paper indicate that the naturally short days are, at least in part, responsible for the failure of

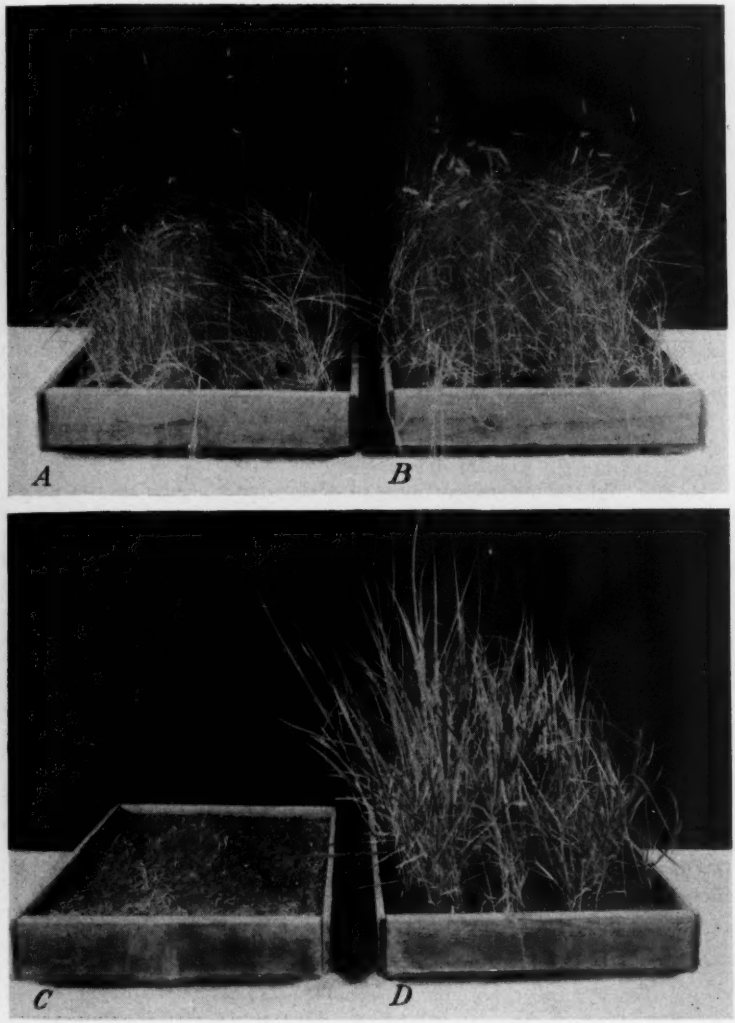

FIGURE 2.—Plants grown for 11 weeks at 75° F.: *Bouteloua gracilis* grown (*A*) in a 10-hour day and (*B*) in an 18-hour day; *Panicum virgatum* grown (*C*) in a 10-hour day and (*D*) in an 18-hour day.

plants of *Agropyron smithii* to bloom and of plants of *Andropogon furcatus* and *Panicum virgatum* to grow well in the greenhouse during the winter. This conclusion is based on the fact that plants of *A.*

smithii bloomed and the plants of *A. furcatus* and *P. virgatum* made good growth when the day length was increased by burning incandescent lamps for several hours each night. The results also tend to show that failure of plants of *Bouteloua gracilis* to flower may have been due to too low temperatures, since the plants of this species bloomed in both long and short days when the temperature was maintained at 75° F. but failed to bloom when the temperature was reduced to 60° for a few hours during the night.

SUMMARY

Plants of *Agropyron smithii* Rydb., *Andropogon furcatus* Muhl., *Bouteloua gracilis* (H. B. K.) Lag., and *Panicum virgatum* L. were grown in the greenhouse under different conditions of day length and temperature. Records were taken on the number of days between planting and the appearance of the first bloom, on the dry weights, and on the root-top ratios of the plants growing under the different conditions.

The results on the number of days required by the plants to flower indicate that *Agropyron smithii* is a long-day plant, *Bouteloua gracilis* is a plant of indeterminate day length, and *Andropogon furcatus* and *Panicum virgatum* are short-day plants.

The dry weights of the plants of *Agropyron smithii* were not affected by the day length, but in the other species the plants growing in the long days had the greater dry weight.

A low night temperature increased the dry weights of the plants of *Agropyron smithii* and *Andropogon furcatus* in both long and short days and also increased the dry weights of the plants of *Panicum virgatum* growing in a short day, but it decreased the dry weights of the plants of *Bouteloua gracilis* growing in both day lengths and also decreased that of plants of *P. virgatum* growing in a long day.

The root-top ratios of the plants of *Andropogon furcatus* and *Panicum virgatum* were greater in the short days than in the long days. No marked difference between the ratios of the plants of the other two species growing in the different day lengths was observed.

There was no significant difference between the root-top ratios of the plants of the four species when grown in a low night temperature and in a high night temperature in either day length.

The results, in general, indicate that day length and temperature markedly affect the growth and flower production of these four grasses and that good growth and blooming can be obtained in the greenhouse in the winter by properly controlling, among other things, these two factors.

LITERATURE CITED

(1) Adams, J.
1923. The effect on certain plants of altering the daily period of light. Ann. Bot. [London] 37: [75]–94.

(2) ———
1924. Duration of light and growth. Ann. Bot. [London] 38: [509]–523.

(3) ———
1924. Does light determine the date of heading out in winter wheat and winter rye? Amer. Jour. Bot. 11: 535–539.

(4) GARNER, W. W., and ALLARD, H. A.
1920. EFFECT OF THE RELATIVE LENGTH OF DAY AND NIGHT AND OTHER FACTORS OF THE ENVIRONMENT ON GROWTH AND REPRODUCTION IN PLANTS. Jour. Agr. Res. 18: 553–606, illus.
(5) ——— and ALLARD, H. A.
1923. FURTHER STUDIES IN PHOTOPERIODISM, THE RESPONSE OF THE PLANT TO RELATIVE LENGTH OF DAY AND NIGHT. Jour. Agr. Res. 23: 871–920, illus.
(6) ——— and ALLARD, H. A.
1931. DURATION OF THE FLOWERLESS CONDITION OF SOME PLANTS IN RESPONSE TO UNFAVORABLE LENGTHS OF DAY. Jour. Agr. Res. 43: 439–443, illus.
(7) GILBERT, BASIL E.
1926. INTERRELATION OF RELATIVE DAY LENGTH AND TEMPERATURE. Bot. Gaz. 81: 1–24, illus.
(8) THOMPSON, H. C.
1933. TEMPERATURE AS A FACTOR AFFECTING FLOWERING OF PLANTS. Amer. Soc. Hort. Sci. Proc. 30: 440–446.
(9) TINCKER, M. A. H.
1925. THE EFFECT OF LENGTH OF DAY UPON THE GROWTH AND REPRODUCTION OF SOME ECONOMIC PLANTS. Ann. Bot. [London] 39: [721]–754, illus.
(10) ———
1928. THE EFFECT OF LENGTH OF DAY UPON THE GROWTH AND CHEMICAL COMPOSITION OF THE TISSUES OF CERTAIN ECONOMIC PLANTS. Ann. Bot. [London] 42: [101]–140, illus.

DIGESTIBLE NUTRIENTS OF FEEDING STUFFS FOR THE DOMESTIC RABBIT [1]

By LEROY VORIS, *assistant professor*, LAWSON F. MARCY, *instructor*, EDWARD J. THACKER, *assistant*, and WALTER W. WAINIO, *instructor*, *Insititute of Animal Nutrition, Pennsylvania Agricultural Experiment Station* [2]

INTRODUCTION

The rational feeding of the domestic rabbit has come to be a matter of consequence since the production of this animal as a source of meat for human consumption has attained a permanent and justifiable place in the general food economy.

This report presents the results of the determination of the composition, digestibility, and digestible nutrients of 47 feeding stuffs available for rabbit feeding in the United States. The results of similar investigations by Weiske (*6, 7, 8*),[3] Von Knieriem (*3, 4*), and Brüggemann (*1*), covering an aggegate of 16 roughages and 12 concentrates, are also included for comparative purposes and for the presentation of digestible-nutrient values not given in, but computed from, the papers by Weiske and Von Knieriem.

EXPERIMENTAL SUBJECTS AND EQUIPMENT

The experimental rabbits were young female New Zealand Whites, weighing about 4 pounds each.

The metabolism cages were a standard product having floor dimensions of 18 by 20 inches and a height of 12 inches. The floor of each cage was of ¾-inch mesh No. 12 gage wire, through which the feces dropped onto a ⅛-inch mesh stainless-steel screen, and the urine was directed by a galvanized-iron funnel pan into a bottle below the cage. The stainless-steel screen and funnel pan were free from the body of the cage and were readily removable for the collection of the excreta.

Water was provided outside the cage proper in a cup which fitted over an opening in the door of the cage.

The usual earthenware feeding dishes were found unsatisfactory for quantitative work, since the feed could be scratched out easily. To overcome this difficulty a galvanized-iron feeder with inbent edges was devised and suspended from the door of the cage. This device almost completely prevented the scattering of feed.

A piece of block salt was kept in the cages of the rabbits at all times.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 683.

EXPERIMENTAL METHODS

FEEDING TECHNIQUE

Roughages were cut with a clover cutter and were shaken through a ¾-inch-mesh screen. The longer pieces thus separated out were returned to the cutter until practically all the material had passed through the screen. The final product was coarse enough to be acceptable to the rabbits, but was relatively free of pieces over 2 inches in length, which they would have wasted.

All of the dry roughages, except bluegrass, were fed alone and the digestibility determined directly. Since the rabbits lost much weight when fed on dried bluegrass alone, this was fed with a commercial rabbit ration and the digestibility determined by difference.

The quantities in which the dry roughages were fed were determined largely by the palatability of the product. The maximum amount of the most satisfactory roughages, such as alfalfa hay, that a rabbit could be trusted to clean up was about 80 gm. per day. In general, the quantity of dry roughage that a rabbit would eat was well below that which would have been required to maintain its body weight.

The green roughages, roots, and tubers were cut up into pea-sized pieces and were fed fresh in quantities estimated to furnish dry matter equivalent to the 40 gm. of alfalfa hay with which they were given.

Aliquot samples were taken at the time of each feeding and were kept in pyrex dishes at 50° C. in a Freas oven. At the end of an experiment the oven-dry samples were allowed to come to moisture equilibrium with the air. They were then weighed, ground in a Wiley mill, and retained for analysis, sealed in glass bottles.

Each of the concentrates was fed with an equivalent weight of alfalfa hay. Cottonseed cake was fed also with soybean hay and with vetch hay. The reasons for this will be given later.

Corn (maize) was fed coarsely cracked; all other grains were fed whole.

The oil cakes were fed in pea size, since rabbits do not like pulverized dry feeds.

Milk was fed in liquid form to the extent of 300 gm. daily with 40 gm. of alfalfa hay. No difficulty was encountered in getting the rabbits to drink 150 gm. of milk at each feeding.

The quantity of concentrate fed was usually 40 gm. daily, in addition to the 40 gm. of alfalfa hay. This combination provided nutriment somewhat in excess of the maintenance requirement.

The feed was given twice daily in equal portions and weighed immediately prior to feeding. Aliquot portions of the concentrates were saved each time the feeding stuffs were weighed, and the composite samples representing the collection period were ground and sealed up in the same way as were the roughage samples.

In general, three rabbits were used for each treatment. That this number was sufficient to establish valid digestion coefficients for the group was determined from experiments in which 10 or more trials were made.

COLLECTION AND TREATMENT OF FECES

The feces were usually collected over a period of 10 days. In certain cases when the supply of feed was insufficient or the animals

showed signs of malaise, the collection periods were curtailed to 7 days. Collection was always preceded by a preliminary feeding period of 5 to 7 days, usually the latter. The preliminary feeding was deemed to be sufficiently long to establish equilibrium between the feeding treatment and any effects of coprophagy that might have existed, since the quantity of feed eaten daily in two equal portions was constant and the daily weights of the feces were consistent after a few days.

Feces were collected daily throughout the experimental periods. The fresh feces were kept overnight at 50° C. in a ventilated Freas oven, from which they were transferred to open jars and left exposed to the air until the end of the collection period. They were then weighed each day during several days, to assure equilibrium with the air, and were then ground in a Wiley mill. The ground samples were kept in sealed bottles for analysis.

METHODS OF ANALYSIS

The feeds and feces were subjected to the conventional analyses for moisture, nitrogen, ether extract, crude fiber, ash, and energy, with nitrogen-free extract computed, as usual, by difference.

Moisture was determined as the loss in weight of a 2-gm. sample when heated overnight at 80° C. in a Freas oven and thereafter kept in a vacuum desiccator containing sulfuric acid until no further loss in weight occurred. Constant weight was obtained after 2 days in the vacuum desiccator. This procedure was found to be quite as accurate as drying to constant weight entirely in the vacuum desiccator, which would have required about 3 weeks.

Nitrogen was determined by the boric acid modification of the Kjeldahl method. Copper sulfate was used as the catalyst in the digestion, and methyl red as the indicator in the titration.

Ether extract was determined by extracting the dried samples employed in the moisture determination for 48 hours in Soxhlet extractors.

Crude fiber was determined on the residue from the ether extraction by the official method of the Association of Official Agricultural Chemists.

Ash was determined as the ignition residue from a 3- to 5-gm. sample which had been brought up slowly, overnight, to a temperature of 550° C. in a muffle furnace under rheostat control.

Energy was determined by means of an adiabatic Emerson bomb calorimeter.

RESULTS

DIGESTIBILITY AND DIGESTIBLE NUTRIENTS OF FEEDING STUFFS

The composition and the digestibility of the feeding stuffs, and the digestible nutrients contained therein, are presented in tables 1, 2, and 3, respectively. Supplementary tables 4, 5, 6, and 7 give the results of the similar work by Weiske (*6*, *7*, *8*), Von Knieriem (*3*, *4*), and Brüggemann (*1*). The feeding stuffs on which results are reported both in the previously published studies referred to and in the investigation now presented are the following: Clover, timothy and vetch hays, barley, linseed cake, milk, oats, rye, and wheat. In general, the digestion coefficients given in the earlier and in the present work are in essential agreement.

A comparison of the results obtained for dry roughages with rabbits with those reported by Morrison (*2*) for similar feeds with ruminants shows, respectively, the following average digestibility: Protein, 65 and 60 percent; ether extract, 48 and 55; crude fiber, 17 and 53; and nitrogen-free extract, 58 and 65 percent.

TABLE 1.—*Composition of feeding stuffs supplied rabbits in this investigation*

Feeding stuff and source	Water	Crude protein	Ether extract	Carbohydrates		Ash	Energy per gram
				Crude fiber	Nitrogen-free extract		
Dry roughages:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Calories*
Alfalfa hay (Pennsylvania)	11.4	17.9	1.5	30.9	32.4	5.9	4.12
Alfalfa hay, western, 1938, (California)	6.5	20.4	2.2	20.4	42.1	8.4	4.20
Alfalfa hay, western, 1939, (California)	10.1	27.2	2.9	21.6	28.8	9.4	4.03
Bluegrass (California)	9.6	26.1	4.3	18.9	31.3	9.8	4.13
Clover hay (Pennsylvania)	12.5	12.9	2.7	24.7	41.2	6.0	3.91
Kale (California)	10.6	23.1	3.7	11.9	33.3	17.4	3.32
Kudzu hay (Alabama)	7.2	10.9	1.5	42.2	33.2	5.0	4.22
Lespedeza, common (Alabama)	10.5	14.8	2.8	27.7	39.0	5.2	4.29
Lespedza sericea (Alabama))	7.9	11.4	1.6	29.7	44.4	5.0	4.29
Lespedza sericea (Mississippi)	11.4	9.4	2.1	32.4	39.8	4.9	4.16
Melilotus hay (California)	10.6	21.1	2.9	22.6	33.0	9.8	3.99
Milo hay, green (California)	10.0	19.3	3.7	21.6	34.2	11.2	3.87
Oat hay, green (Pennsylvania)	6.8	7.1	3.1	37.4	38.3	7.3	4.10
Peanut hay (Alabama)	7.9	9.7	2.6	37.7	37.5	4.6	4.14
Soybean hay (Alabama)	10.4	10.6	1.4	38.0	33.2	6.4	3.95
Soybean hay, green (Pennsylvania)	7.5	14.5	1.9	28.6	37.5	10.0	3.94
Sudan grass (California)	11.0	15.8	3.7	20.2	40.5	8.8	3.88
Timothy hay (Pennsylvania)	10.1	5.9	1.8	30.7	47.1	4.4	4.07
Vetch hay (California)	10.3	19.2	3.1	24.5	35.3	7.6	3.96
Wheat hay, green (Pennsylvania)	10.3	19.4	3.6	23.2	33.7	9.8	3.93
Green roughages, roots, tubers:							
Cabbage	91.5	1.7	.1	.9	5.1	.7	.34
Carrot	87.7	1.4	.1	1.2	8.7	.9	.48
Celery	94.4	.9	.1	.8	2.5	1.3	.19
Rutabaga	86.9	1.3	.1	1.2	9.8	.7	.52
Sweetpotato	58.1	1.8	.3	1.0	37.9	.9	1.73
Turnip	91.8	1.0	.1	1.1	5.2	.8	.32
Yam	68.6	1.4	.4	.9	27.7	1.0	1.29
Concentrates:							
Barley	14.5	10.7	1.2	6.4	65.0	2.2	3.88
Beet pulp	11.5	8.3	.3	21.8	55.5	2.6	3.83
Bread, dried	4.4	15.8	2.5	.3	73.5	3.5	4.23
Brewers' grains	5.7	28.7	5.8	16.2	40.6	3.0	4.93
Buckwheat	14.8	10.4	2.3	10.8	60.0	1.7	3.85
Corn	14.0	10.0	3.6	2.1	68.9	1.4	3.98
Cottonseed-oil cake	6.7	39.7	6.6	13.3	26.8	6.9	4.55
Linseed-oil cake	10.8	36.8	4.0	7.8	35.2	5.4	4.29
Milk	87.8	3.1	3.7	------	4.6	.8	.72
Milo maize	6.5	12.1	2.8	1.9	75.0	1.7	4.03
Oats	12.1	10.9	4.2	10.6	58.9	3.3	4.18
Peanut-oil cake	6.0	42.8	7.7	4.4	32.6	6.5	4.57
Rye	14.1	9.7	1.4	2.1	70.9	1.8	3.91
Sesame-oil cake	5.5	39.4	8.7	6.7	26.5	13.2	4.22
Soybean-oil cake	9.3	45.5	4.6	5.0	29.9	5.7	4.44
Soybeans, whole, Manchu	6.8	40.9	17.1	5.6	24.5	5.1	5.23
Wheat	14.9	8.9	1.3	2.2	70.9	1.8	3.79
Wheat bran	10.4	16.7	3.9	10.5	51.6	6.9	4.09
Mixed rations:							
Purina omolene	12.1	12.4	2.7	7.8	60.6	4.4	3.98
Purina rabbit chow	11.0	14.9	3.3	13.7	52.1	5.0	4.02

TABLE 2.—*Average digestibility of feeding stuffs supplied rabbits in this investigation*

Feeding stuff and source	Trials	Dry matter	Crude protein	Ether extract	Carbohydrates		Ash	Energy
					Crude fiber	Nitrogen-free extract		
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Dry roughages:								
Alfalfa hay (Pennsylvania)	19	48.1	72.3	16.0	18.2	63.3	62.4	45.9
Alfalfa hay, western, 1938 (California)	3	67.5	80.7	20.0	29.8	81.9	67.4	64.6
Alfalfa hay, western, 1939 (California)	3	63.4	83.6	39.1	27.3	74.8	60.7	61.6
Bluegrass (California)	3	45.2	74.4	40.9	12.6	41.0	45.7	45.4
Clover hay (Pennsylvania)	3	52.8	62.8	61.8	19.7	67.2	64.3	49.3
Kale (California)	3	76.0	81.1	40.7	59.5	83.4	74.1	69.3
Kudzu hay (Alabama)	3	38.0	62.5	12.4	16.1	55.8	59.0	34.4
Lespedeza, common (Alabama)	3	44.7	66.7	45.9	10.6	59.3	53.1	43.0
Lespedeza sericea (Alabama)	3	31.5	28.7	54.3	7.3	46.5	41.4	28.6
Lespedeza sericea (Mississippi)	3	32.9	30.8	50.0	8.5	50.2	51.1	30.5
Melilotus hay (California)	3	56.9	79.0	49.2	6.5	73.4	72.4	53.6
Milo hay, green (California)	2	44.8	71.1	46.3	15.7	46.6	49.5	42.7
Oat hay, green (Pennsylvania)	1	29.1	61.0	53.8	10.4	35.5	50.0	26.9
Peanut hay (Alabama)	3	47.1	55.0	69.7	25.0	65.9	46.1	44.2
Soybean hay (Alabama)	1	41.3	67.1	31.8	16.6	58.9	54.6	38.1
Soybean hay, green (Pennsylvania)	3	52.3	69.6	9.0	24.0	71.7	43.6	49.6
Sudan grass (California)	3	53.8	68.1	49.4	26.6	64.1	44.9	52.3
Timothy hay (Pennsylvania)	3	36.5	46.6	44.2	10.7	51.0	45.3	34.6
Vetch hay (California)	3	56.2	78.3	62.8	11.0	72.4	68.5	52.6
Wheat hay, green (Pennsylvania)	3	50.5	77.6	50.3	22.0	53.2	55.7	49.3
Green roughages, roots, tubers:								
Cabbage	3	101.3	98.6	83.3	88.2	102.9	94.1	100.3
Carrot	3	92.8	85.7	79.4	56.4	97.8	87.6	90.8
Celery	3	92.5	76.8	90.7	93.3	98.8	86.6	89.3
Rutabaga	3	99.3	89.4	83.3	103.6	100.0	96.0	97.6
Sweetpotato	2	92.6	43.8	75.6	94.3	94.4	103.7	90.4
Turnip	3	97.7	90.6	102.9	82.1	101.0	86.2	96.7
Yam	3	96.1	51.7	80.2	123.1	96.8	109.0	93.7
Concentrates:								
Barley	3	82.5	84.8	106.2	12.5	89.3	41.1	82.4
Beet pulp	3	82.1	48.3	−52.4	71.8	91.6	77.7	79.1
Bread, dried	3	100.2	95.2	98.5	------	100.9	105.4	99.0
Brewers' grains	3	56.4	84.7	80.8	20.6	48.7	15.6	60.0
Buckwheat	3	78.7	72.2	95.2	16.5	89.3	89.2	76.1
Corn	10	91.3	84.2	93.1	145.8	91.9	44.8	92.0
Cottonseed-oil cake	4	66.0	83.5	98.6	30.7	56.3	33.6	70.4
Linseed-oil cake	3	77.3	86.4	98.8	20.5	81.1	34.6	79.4
Milk	3	92.3	99.6	95.3	------	94.1	84.1	91.1
Milo maize	3	89.3	72.1	95.6	103.1	90.6	105.8	86.9
Oats	3	68.2	78.6	97.6	−2.4	78.7	20.7	69.6
Peanut-oil cake	3	91.4	91.2	101.1	48.9	96.2	69.2	92.3
Rye	3	90.5	79.1	82.1	53.6	93.0	86.2	89.7
Sesame-oil cake	3	81.6	90.9	101.3	73.2	84.4	30.2	90.3
Soybean-oil cake	4	89.7	89.7	96.1	51.9	95.5	66.8	90.0
Soybeans, whole, Manchu	3	88.8	87.9	95.5	85.3	90.8	62.9	89.4
Wheat	3	93.3	84.6	100.0	28.5	96.6	53.6	92.8
Wheat bran	3	62.6	83.0	77.2	24.3	65.3	33.0	64.3
Mixed rations:								
Purina omolene	6	74.9	76.3	87.2	17.0	82.8	52.7	74.6
Purina rabbit chow	5	68.1	76.3	81.0	18.2	78.9	58.8	67.2

TABLE 3.—*Dry-matter content and digestible nutrients of feeding stuffs supplied rabbits in this investigation*

Feeding stuff and source	Total dry matter	Digestible nutrients in—					
		Crude protein	Carbohydrates			Fat	Total
			Crude fiber	Nitrogen-free extract	Total		
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Dry roughages:							
Alfalfa hay (Pennsylvania)	88.6	12.9	5.6	20.5	26.1	0.5	39.5
Alfalfa hay, western, 1938 (California)	93.5	16.5	6.1	34.5	40.6	1.0	58.1
Alfalfa hay, western, 1939 (California)	89.9	22.7	5.9	21.5	27.4	2.5	52.6
Bluegrass (California)	90.4	19.4	2.4	12.8	15.2	4.0	38.5
Clover hay (Pennsylvania)	87.5	8.1	4.9	27.7	32.6	3.8	44.6
Kale (California)	89.4	18.7	7.1	27.8	34.9	3.4	57.0
Kudzu hay (Alabama)	92.8	6.8	6.8	18.5	25.3	.4	32.5
Lespedeza, common (Alabama)	89.5	9.9	2.9	23.1	26.0	2.9	38.8
Lespedeza sericea (Alabama)	92.1	3.3	2.2	20.6	22.8	2.0	28.1
Lespedeza sericea (Mississippi)	88.6	2.9	2.8	20.0	22.8	2.4	28.1
Melilotus hay (California)	89.4	16.7	1.5	24.2	25.7	3.2	45.6
Milo hay, green (California)	90.0	13.7	3.4	15.9	19.3	3.8	36.8
Oat hay, green (Pennsylvania)	93.2	4.3	3.9	13.6	17.5	3.8	25.6
Peanut hay (Alabama)	92.1	5.3	9.4	24.7	34.1	4.1	43.5
Soybean hay (Alabama)	89.6	7.1	6.3	19.6	25.9	1.0	34.0
Soybean hay, green (Pennsylvania)	92.5	10.1	6.9	26.9	33.8	.4	44.3
Sudan grass (California)	89.0	10.8	5.4	26.0	31.4	4.1	46.3
Timothy hay (Pennsylvania)	89.9	2.7	3.3	24.0	27.3	1.8	31.8
Vetch hay (California)	89.7	15.0	2.7	25.6	28.3	4.4	47.7
Wheat hay, green (Pennsylvania)	89.7	15.1	5.1	17.9	23.0	4.1	42.2
Green roughages, roots, and tubers:							
Cabbage	8.5	1.7	.8	5.1	5.9	.2	7.8
Carrot	12.3	1.2	.7	8.5	9.2	.2	10.6
Celery	5.6	.7	.7	2.5	3.2	.2	4.1
Rutabaga	13.1	1.2	1.2	9.8	11.0	.2	12.4
Sweetpotato	41.9	.8	.9	35.8	36.7	.5	38.0
Turnip	8.2	.9	.9	5.2	6.1	.2	7.2
Yam	31.4	.7	.9	26.8	27.7	.7	29.1
Concentrates:							
Barley	85.5	9.1	.8	58.0	58.8	2.7	70.6
Beet pulp	88.5	4.0	15.7	50.8	66.5	.0	70.5
Bread, dried	95.6	15.0	.0	73.5	73.5	5.5	94.0
Brewers' grains	94.3	24.3	3.3	19.8	23.1	10.5	57.9
Buckwheat	85.2	7.5	1.8	53.6	55.4	4.9	67.8
Corn	86.0	8.4	2.1	63.3	65.4	7.5	81.3
Cottonseed-oil cake	93.3	33.1	4.1	15.1	19.2	14.6	66.9
Linseed-oil cake	89.2	31.8	1.6	28.5	30.1	8.9	70.8
Milk	12.2	3.1	------	4.3	4.3	7.9	15.3
Milo maize	93.5	8.7	1.9	68.0	69.9	6.0	84.6
Oats	87.9	8.6	.0	46.4	46.4	9.2	64.2
Peanut-oil cake	94.0	39.0	2.2	31.4	33.6	17.3	89.9
Rye	85.9	7.7	1.1	65.9	67.0	2.6	77.3
Sesame-oil cake	94.5	35.8	4.9	22.4	27.3	19.6	82.7
Soybean-oil cake	90.7	40.8	2.6	28.6	31.2	9.9	81.9
Soybeans, whole, Manchu	93.2	36.0	4.8	22.2	27.0	36.7	99.7
Wheat	85.1	7.5	.6	68.5	69.1	2.9	79.5
Wheat bran	89.6	13.9	2.6	33.7	36.3	6.8	57.0
Mixed rations:							
Purina omolene	87.9	9.5	1.3	50.2	51.5	5.3	66.3
Purina rabbit chow	89.0	11.4	2.5	41.1	43.6	6.0	61.0

TABLE 4.—*Composition of feeding stuffs supplied rabbits in investigations of Von Knieriem and Weiske*

Feeding stuff	Water	Crude protein	Ether extract	Carbohydrates		Ash	Reference
				Crude fiber	Nitrogen-free extract		
Dry roughages:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Barbarea vulgaris hay	13.7	14.3	3.1	28.2	34.1	6.6	Von Knieriem (*3, 4*).
Clover hay, white	13.4	16.0	3.8	17.2	40.5	9.1	Do.
Clover hay, red	13.3	13.5	4.0	24.3	39.3	5.6	Do.
Darnel grass hay	8.3	6.3	2.7	36.0	42.4	4.3	Do.
Geum rivale hay	12.5	8.9	3.4	23.4	43.3	8.5	Do.
Kidney-vetch hay	11.8	10.3	3.2	28.9	39.5	6.3	Do.
Orchard grass, rank	11.8	10.2	2.8	28.1	41.0	6.1	Do.
Orchard grass, spare	11.4	6.8	2.2	29.2	43.5	6.9	Do.
Timothy hay	10.3	6.5	1.6	36.9	39.6	5.1	Do.
Vetch hay	15.3	15.5	2.8	23.4	32.3	10.7	Do.
Concentrates:							
Barley	6.9	11.3	2.0	4.2	71.9	3.7	Weiske (*8*).
Coconut cake	12.0	21.8	13.5	14.4	33.0	5.3	Von Knieriem (*3, 4*).
Hempseed cake	13.7	26.1	9.2	29.9	12.6	8.5	Do.
Linseed cake	11.9	30.1	9.0	9.2	33.2	6.6	Do.
Milk	90.7	3.5	.5	-------	4.5	.8	Do.
Oats	6.5	11.1	5.5	9.3	64.7	2.9	Weiske (*6, 7*).
Palm-kernel cake	10.5	16.9	12.0	17.4	39.0	4.2	Von Knieriem (*3, 4*).
Rapeseed cake	10.6	29.9	8.5	11.9	32.1	7.0	Do.
Rye	6.0	12.0	1.7	2.1	76.3	1.9	Weiske (*8*).
Sunflower cake	9.5	30.7	9.5	19.4	25.8	5.1	Von Knieriem (*3, 4*).

TABLE 5.—*Digestibility of feeding stuffs supplied rabbits in investigations of Von Knieriem and Weiske*

Feeding stuffs	Dry matter	Crude protein	Ether extract	Carbohydrates		Ash	Reference
				Crude fiber	Nitrogen-free extract		
Dry roughages:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Barbarea vulgaris hay	55.3	77.9	62.4	25.9	66.3	72.3	Von Knieriem (*3, 4*).
Clover hay, white	72.7	68.2	50.9	57.4	83.1	72.4	Do.
Clover hay, red	55.1	64.4	75.3	26.5	68.2	53.6	Do.
Darnel grass hay	35.4	54.2	57.2	12.5	51.8	22.7	Do.
Geum rivale hay	55.2	32.9	62.0	25.6	73.1	66.5	Do.
Kidney-vetch hay	55.9	65.8	60.1	27.1	73.5	59.6	Do.
Orchard grass, rank	47.6	76.0	64.5	15.2	58.5	68.5	Do.
Orchard grass, spare	44.2	71.8	63.1	12.4	59.4	55.9	Do.
Timothy hay	35.6	56.7	55.0	18.5	48.2	27.4	Do.
Vetch hay	56.7	71.3	58.0	29.9	69.2	56.3	Do.
Concentrates:							
Barley	84.0	67.7	86.3	25.1	91.2	51.2	Weiske (*8*).
Coconut cake	94.4	95.7	99.1	89.1	95.2	86.6	Von Knieriem (*3, 4*).
Hempseed cake	-------	65.2	90.1	29.3	8.2	11.3	Do.
Linseed cake	-------	86.0	93.4	28.1	76.0	38.7	Do.
Milk	-------	97.7	91.7	-------	97.8	80.0	Do.
Oats	73.7	80.2	93.8	21.6	79.5	46.4	Weiske (*8*).
Palm-kernel cake	-------	92.8	97.7	64.0	77.9	-------	Von Knieriem (*3, 4*).
Rapeseed cake	-------	78.9	85.4	39.8	73.2	-------	Do.
Rye	84.4	63.0	76.3	18.5	91.2	34.4	Weiske (*8*).
Sunflower cake	-------	85.7	79.1	13.7	45.0	-------	Von Knieriem (*3, 4*).

TABLE 6.—*Dry-matter content and digestible nutrients of feeding stuffs supplied rabbits as computed from data of Von Knieriem and Weiske*

Feeding stuff	Total dry matter	Digestible nutrients in—						Reference
		Crude protein	Carbohydrates			Fat	Total	
			Crude fiber	Nitrogen-free extract	Total			
Dry roughages:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Barbarea vulgaris hay	86.3	11.1	7.3	22.6	29.9	4.4	45.4	Von Knieriem (3, 4).
Clover hay, white	86.6	10.9	9.9	33.7	43.6	4.4	58.9	Do.
Clover hay, red	86.7	8.7	6.4	26.8	33.2	6.8	48.7	Do.
Darnel grass hay	91.7	4.0	4.5	22.0	26.5	3.5	34.0	Do.
Geum rivale hay	87.5	2.9	6.0	31.7	37.7	4.7	45.3	Do.
Kidney-vetch hay	88.2	6.8	7.8	29.0	36.8	4.3	47.9	Do.
Orchard grass rank	88.2	7.8	4.3	24.0	28.3	4.1	40.2	Do.
Orchard grass, spare	88.6	4.9	3.6	25.8	29.4	3.1	37.4	Do.
Timothy hay	89.7	3.7	6.8	19.1	25.9	2.0	31.6	Do.
Vetch hay	84.7	11.1	7.0	22.4	29.4	3.7	44.2	Do.
Concentrates:								
Barley	93.1	7.7	1.1	65.6	66.7	3.9	78.3	Weiske (8).
Coconut cake	88.0	20.9	12.8	31.4	44.2	30.1	95.2	Von Knieriem (3, 4).
Hempseed cake	86.3	17.0	8.8	1.0	9.8	18.7	45.5	Do.
Linseed cake	88.1	25.9	2.6	25.2	27.8	18.9	72.6	Do.
Milk	9.3	3.4	.....	4.5	4.5	1.0	8.9	Do.
Oats	93.5	8.9	2.0	51.4	53.4	11.6	73.9	Weiske (8).
Palm-kernel cake	89.5	15.7	11.1	30.4	41.5	26.4	83.6	Von Knieriem (3, 4).
Rapeseed cake	89.4	23.6	4.7	23.5	28.2	16.3	68.1	Do.
Rye	94.0	7.6	1.4	69.6	71.0	2.9	81.5	Weiske (8).
Sunflower cake	90.5	26.3	2.7	11.6	14.3	16.9	57.5	Von Knieriem (3, 4).

TABLE 7.—*Digestibility and digestible nutrients of feeding stuffs supplied rabbits as computed from data of Brüggemann (1)*

Feeding stuff	Digestibility of—					Digestible nutrients	
	Organic matter	Crude protein	Crude fat	Crude fiber	Nitrogen-free extract	Protein	Tota
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Meadow hay	47.2	60.1	43.1	18.5	64.5	5.2	39.1
Lucerne hay	61.3	75.7	15.9	38.6	72.6	16.6	48.5
Stinging nettle hay	63.0	95.6	31.6	42.4	78.9	20.8	51.9
Green lucerne	81.5	89.1	70.8	66.0	88.0	5.6	15.8
Green sweet lupine	78.9	91.1	73.2	56.5	84.1	3.3	8.9
Ensilaged maize	55.0	79.4	96.1	24.2	68.8	1.3	12.7
Oats	63.8	79.0	90.3	18.0	72.2	10.3	57.1
Barley	89.6	78.0	90.2	72.2	93.3	9.7	77.2
Wheat	94.0	81.0	91.8	90.5	97.0	10.9	82.2
Beet roots	96.6	66.4	.....	100.0	95.7	.3	10.9
Steamed potatoes	98.3	58.3	100.0	100.0	99.1	1.3	22.0

It is noticeable from this comparison that the digestibility of the crude fiber of dry roughages by rabbits is remarkably low. This observation is not new, having been made by Von Knieriem as early as 1898 and recently confirmed by Brüggemann (*1*) and Watson and Godden (*5*). The latter workers found the total digestibility, as well as that of crude fiber, of pasture herbage, consisting of mixed grasses with some clover, to be less with rabbits than with sheep. They suggested that the lower digestibility of crude protein, ether extract, and nitrogen-free extract might well be due to sweeping-out or occlusion effects of the indigestible fiber. Such effects are not evident from the present data, since the digestibility of the constituents other than crude fiber compares fairly well with that for ruminants.

From the data reported by Morrison (*2*) for horses and swine it appears that the capacity of rabbits to digest crude fiber is less than that of horses and nearly equivalent to that of swine.

Since the digestibility of true fat by rabbits is almost complete, as evidenced by the digestibility of the ether extracts from the whole grains, seeds, and oil cakes, it seems quite justifiable to suggest that the percentage digestibility of the ether extracts from the dry roughages furnishes a rough measure of the proportion of the fatty acid esters to the nonlipide constituents of the ether extracts.

Among the better roughages for rabbits, judging by the digestible nutrients they furnish, are dried kale, alfalfa, vetch, and clover hay, green soybean and green wheat hay, and Sudan grass. Peanut hay might be included in this list, but the lot of hay employed in the present work contained some nuts, which may have accounted for a considerable portion of the digestible nutrients.

The poorest of the dry roughages was definitely oat hay. Not only was it unpalatable but the weight of the feces from the hay almost equaled that of the hay itself. One rabbit died, apparently of undernutrition, after subsisting on 60 gm. of oat hay daily for 9 days. Another rabbit, subsisting on 80 gm. of oat hay daily, lost 560 gm., or 30 percent of its original weight, in 2 weeks.

A noticeable difference in palatability was evident between the *Lespedeza sericea* from Mississippi and that from Alabama. The former had a pronounced odor of tea and was much less palatable than the sample from Alabama. The digestibility of the two samples, however, was not considerably different.

All the constituents of the green roughages, roots, and tubers were found highly digestible by rabbits. Even the crude fiber of these feeding stuffs was highly digestible. It is obvious, however, that the method of determination of crude fiber is not sufficiently definitive to allow interpretation of the high digestibility of crude fiber in these feeding stuffs as compared with its low digestibility in the dry roughages. It appears that the chief virtue of the green roughages and roots as feeding stuffs for rabbits is in their accessory food factors rather than in the quantities of digestible nutrients which they supply.

In regard to the concentrates—those which furnished digestible nutrients in excess of 80 percent of their weight are listed in order as follows: Soybeans, dried bread, peanut-oil cake, milo maize, sesame-oil cake, and corn. Wheat, rye, linseed-oil cake, barley, and dried beet pulp furnish digestible nutrients ranging from 70 to 80 percent of their weight. The constituents of milk are highly digestible by rabbits, but this feeding stuff is doubtless more valuable in furnishing accessory food factors than quantities of digestible nutrients. Soybean-oil cake, peanut-oil cake, soybeans, sesame-oil cake, linseed-oil cake, and brewer's grains may be listed as notable sources of protein for rabbits.

TOXICITY OF COTTONSEED-OIL CAKE FOR RABBITS

There seem to be no published observations on the toxicity of cottonseed-oil cake for rabbits. In the early part of this work cottonseed-oil cake was offered in pellet form, with an equal weight of alfalfa hay, to three rabbits. Two of these rabbits refused to eat the cottonseed pellets after the second day. The third rabbit continued to eat the cottonseed for 9 days before refusing. This rabbit

ceased producing feces and lost all appetite for any kind of feed offered. By the eleventh day its abdomen was hard and bloated, and it died on the thirteenth day after cottonseed-oil cake had first been eaten. Post-mortem examination indicated practically complete intestinal stasis. The contents of the caecum and lower gut were hard-packed and dry.

Attempts to feed other rabbits on cottonseed pellets and alfalfa hay ended with their rejection of the cottonseed after 2 or 3 days. No obvious ill effects appeared in these rabbits.

At a later date three rabbits were offered pellets of cottonseed-oil cake with vetch hay. They refused to eat the cottonseed after 6 days, showing signs of constipation. Two of these rabbits recovered after being fed cabbage and a commercial rabbit ration for several days. The third rabbit continued to fail; and, because of the advancing symptoms of anorexia, constipation, abdominal rigidity, etc., it was killed for autopsy. Among the overt symptoms, intestinal stasis was again marked. The lungs, kidney, heart, and liver appeared normal.

The feeding of cottonseed-cake pellets with soybean hay (green, dried, from Pennsylvania) was unique in that the rabbits continued to eat the cottonseed over a much longer period than with the other roughages. One rabbit exhibited no ill effects after receiving 40 gm. of cottonseed cake with 40 gm. of soybean hay daily for 16 days. None of the unfavorable symptoms observed previously in other rabbits appeared in this one; but 4 days after being taken off the cottonseed diet and put onto a commercial rabbit ration the rabbit died. The second of these rabbits ate the cottonseed with soybean hay for 15 days before exhibiting signs of constipation and failure of appetite. This rabbit continued to fail and was killed on the eighteenth day after initiation of the cottonseed feeding. The third rabbit in this group rejected the cottonseed-soybean hay diet after 14 days, lingered for 7 days by nibbling at offerings of cabbage and commercial rabbit ration, but died on the twenty-second day after the initiation of the cottonseed feeding.

The cottonseed cake fed was made from seed grown in the San Joaquin Valley, Calif., in 1937.

No study was made of the cause of the observed toxicity of the cottonseed-oil cake.

SUMMARY

The composition, digestibility, and digestible nutrients of 47 feeding stuffs, including dry roughages, green roughages, roots, tubers, concentrates, and commercial mixed feeds, for rabbits, are reported. The greater part of the determinations were made in triplicate.

Concentrates were found to be well digested, and, with the exception of crude fiber, roughages were nearly as well digested by rabbits as by domestic animals.

Kale, alfalfa, vetch, clover, soybean, wheat, and Sudan grass were found to be among the better roughages for rabbits.

The poorest roughages studied were bluegrass and oat hay.

When fed as the sole concentrate, with the same quantity of roughage, cottonseed meal was found to be toxic to rabbits, the most prominent effects being intestinal stasis and loss of appetite.

Results of previously published work are tabulated and all available data concerning the utilization of feeding stuffs by rabbits are thus brought together.

LITERATURE CITED

(1) BRÜGGEMANN, HANS.
1939. DIE AUSNUTZUNG EINIGER FUTTERMITTEL DURCH DAS KANINCHEN. 7th World's Poultry Cong., Cleveland, Proc. 1939: 471–473.

(2) HENRY, W. A., and MORRISON, F. B.
1923. FEEDS AND FEEDING; A HANDBOOK FOR THE STUDENT AND STOCKMAN. Ed. 18, rewritten by F. B. Morrison, 770 pp., illus. Madison, Wis.

(3) KNIERIEM, W. v.
1898. VERSUCHE ZUR WIRTSCHÄTZUNG DES WIESENHEUES, AUSGEFÜHRT AUF DER VERSUCHSFARM PETERHOF. Landw. Jahrb. 27: [521]–565.

(4) ———
1898. UNTERSUCHUNGEN BETREFFEND DEN WERT VERSCHIEDENER KRAFTFUTTERMITTEL. Landw. Jahrb. 27: [566]–630.

(5) WATSON, C. J., and GODDEN, W.
1935. THE COMPARATIVE DIGESTIBILITY OF ARTIFICIALLY-DRIED PASTURE HERBAGE BY SHEEP AND RABBITS. Empire Jour. Expt. Agr. 3: 346–350.

(6) WEISKE, H.
1892. UEBER DIE VERDAULICHKEIT DES FUTTERS (HEU, HAFER) UNTER VERSCHIEDENEN UMSTÄNDEN UND BEI VERSCHIEDENEN THIEREN. Landw. Jahrb. 21: [791]–807.

(7) ———
1892. ÜBER DEN EINFLUSS DES VERMEHRTEN ODER VERMINDERTEN FUTTERKONSUMS, SOWIE DER DEM FUTTER BEIGEGEBENEN SALZE AUF DIE VERDAUUNG UND RESORPTION DER NAHRUNGSTOFFE. Landw. Vers. Sta. 41: 145–164.

(8) ———
1894. VERSUCHE ÜBER DIE VERDAULICHKEIT UND DEN NAHRWERT VERSCHIEDENER CEREALIENKÖRNER. Landw. Vers. Sta. 43: 207–222.

CANE GALL OF BRAMBLES CAUSED BY PHYTOMONAS RUBI N. SP.[1]

By E. M. HILDEBRAND

Assistant professor of plant pathology, New York (Cornell) Agricultural Experiment Station

INTRODUCTION

Because of a similarity in gross symptoms, cane gall has often been confused with crown gall caused by *Phytomonas tumefaciens* (Sm. and Town.) Bergey *et al.*, although its identity was apparently established by Banfield (*2, 4, 18*).[2] Pinckard (*13*), in a comparative study of the physiology of cell-stimulating bacteria, demonstrated rather conclusively specific differences between these two organisms and provided a basis for describing the cane gall organism. The present paper summarizes the results of observations made on this disease and its causal organism in New York State during the past 7 years and brings together other pertinent facts from the literature to assist in describing the causal organism.

Cane gall is undoubtedly an old disease since over 40 years ago Bailey (*1*) reported in New York a so-called cane-knot disease of blackberries and illustrated typical cane gall symptoms. He states: "It is apparently not common, but it must be widespread for I have had specimens from as far west as Wisconsin." Shortly thereafter a similar disease was reported from Europe (*17, p. 606, 19, 20*). That cane gall now occurs widely in the raspberry-growing sections of the United States (Ohio, Indiana, Michigan, Illinois, and Oregon) has been indicated by Banfield (*4, 18*). The writer found it in New York in 1932 and Zundel (*21*) has noted its presence in Pennsylvania.

SYMPTOMS

Symptoms appear on the fruiting canes of *Rubus* spp. in late May or June as small spherical protuberances or elongate ridges of white granular gall tissue (fig. 1). The small whitish eruptions rapidly increase in size and number and may completely cover sections of the cane surface, being most abundant on the lower part of the cane but appearing also on the upper part and even on the small terminal branches. After several weeks the whitish gall tissue turns brown and begins to disintegrate near the soil surface (fig. 2). The enlargement of the galls frequently causes the stems to split open and the canes to dry out. These injured canes produce only small seedy berries. Growers refer to the condition as "beading," "coraling," or "knotting."

Under severe disease conditions one New York grower wrote Bailey (*1*), that the disease "progresses rapidly, as the fruit grows, and when the fruit is about two-thirds grown the leaves begin to wither,

[1] Received for publication March 20, 1940.

[2] Italic numbers in parentheses refer to Literature Cited, p. 695.

the cane dries up, and the berries ripen. On very badly diseased canes the berries wither and dry up." The course of the disease as outlined above was very similar to that observed on the Sodus variety of purple raspberry in the writer's garden in 1939.

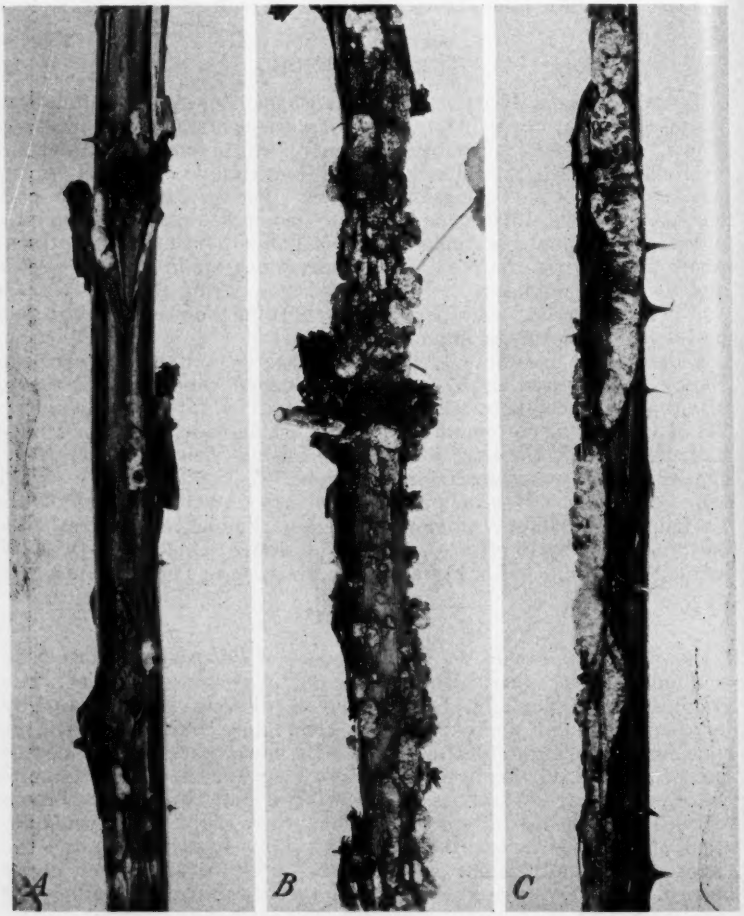

FIGURE 1.—Stages in cane gall development on black raspberry. The first symptoms appear (*A*) on the fruiting canes in May as spheres or ridges of white granular tissue. The galls increase in number and size and during June they may appear singly or in rows (*B*) or in more or less elongate ridges (*C*). (Photographs furnished by W. M. Banfield).

According to Banfield, cane galls are seldom found on the roots of black raspberry, the plant principally used in his studies, or on the roots or canes of red raspberry, and rarely, if ever, on black raspberry canes during the first year of their growth. Bailey (*1*) states that the disease, "probably attacks the growing shoots, although it is

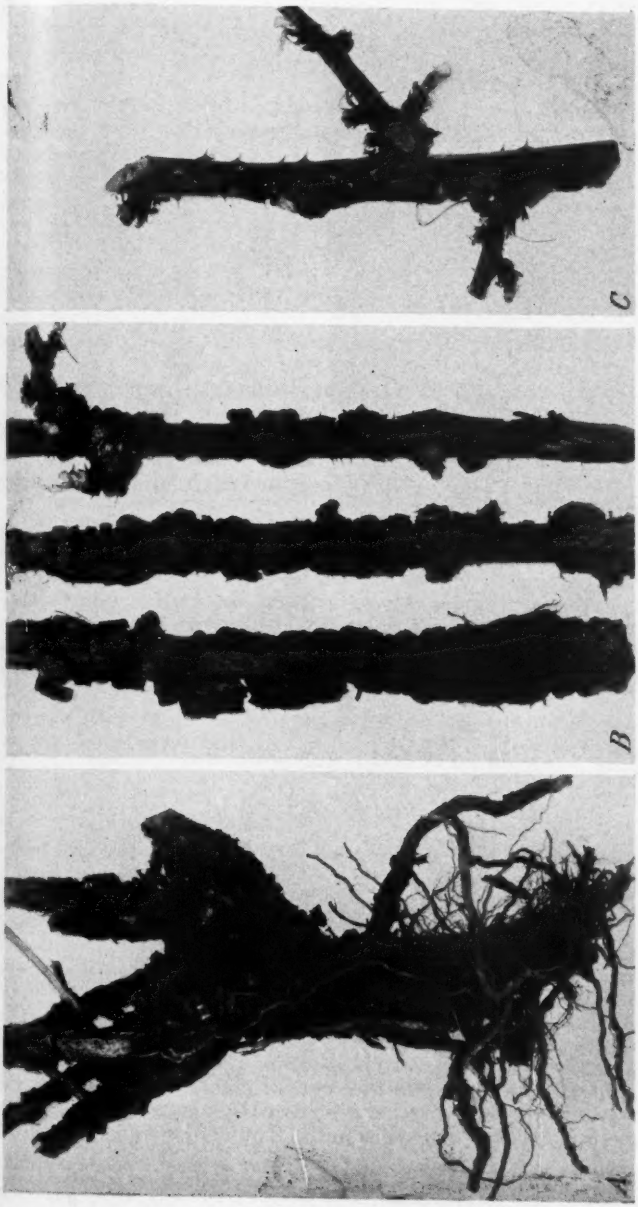

FIGURE 2.—Advanced stage of cane gall on a Plum Farmer black raspberry plant collected in the field in July. Practically all of the galls are discolored and in an advanced stage of deterioration and there is little new cane growth: *A*, Plant showing disintegration of the crown region and sparseness of current season cane growth therefrom; *B*, abundance of galls along the fruiting canes (sometimes these appear in rows and sometimes in indiscriminate order); *C*, galls that have developed on the side branches well up on the plant.

not apparent until the following year, when the grower, noticing that the leaves are yellow and the fruit not filling, examines the canes and finds these knots upon them."

ISOLATION STUDIES

The cane gall organism was readily isolated from fresh young galls by using poured plates of such media as potato-dextrose or potato-mannitol agar.[3] Banfield (*4*) induced cane galls on black raspberry under aseptic conditions, and when isolations were attempted 3 weeks later only cane gall bacteria appeared on the plates. Numerous isolations by the writer from young galls of about the same age, but produced under exposed conditions, generally gave cultures relatively free from contaminants. However, when isolations were attempted from older galls and from soil, serious difficulty was encountered with contaminants, even in the former case, although the greatest care was exercised in removing the outer gall tissue before taking samples.

To discover a more suitable isolation medium, a comparative study was made involving, in addition to the common laboratory media, several selective media (*7*, *11*, *12*) of which the aniline blue medium of Hendrickson et al. proved fairly satisfactory. The bacterial colonies resulting from tissue and soil isolations came up in aniline blue agar plates largely free from contaminants.

Whenever discoloration became pronounced in the cane galls, which was a rather common occurrence in the field in July, it was no longer possible to recover cane gall bacteria from them by any method tried.

Before pathogenicity tests were carried out, the parent cultures were purified by making single-cell isolations (*10*). Five single cells were isolated from each of the original parent cultures obtained from two black and one purple raspberry planting in New York. Growth resulted from two cells isolated from culture 1, five from culture 2, and three from culture 3. These 13 isolates (counting both parents and progeny) appeared identical in culture and were employed in making subsequent studies.

PATHOGENICITY

Galls were readily induced by inoculation of the organism into fruiting canes, current season cane growth and petioles, and roots. Figure 3, *A*, illustrates an early gall development on a current season cane and petiole stimulated by needle-puncture inoculation. A later stage produced in the same manner is shown in figure 3, *B*. A high incidence of infection ranging from 80 to 100 percent usually resulted from needle-puncture inoculations into current-season canes. Figure 3, *C*, illustrates an advanced stage of gall development on new growth, the radial extension of the gall being over 1 inch. Brown patches over the surface indicate that decomposition is taking place.

Small galls were produced on the roots of plants when the bacteria were introduced into the soil either directly or by means of water washing the bacteria off galls above ground. Wounds for infection courts

[3] The ingredients for 1 liter of potato-dextrose agar consisted of 200 gm. of potato (skins removed), 17 gm. of agar, 10 or 20 gm. of dextrose, and distilled water. The same ingredients were used in potato-mannitol agar except for the substitution of 5 gm. of mannitol for the dextrose and the addition of a small pinch of finely powdered calcium carbonate.

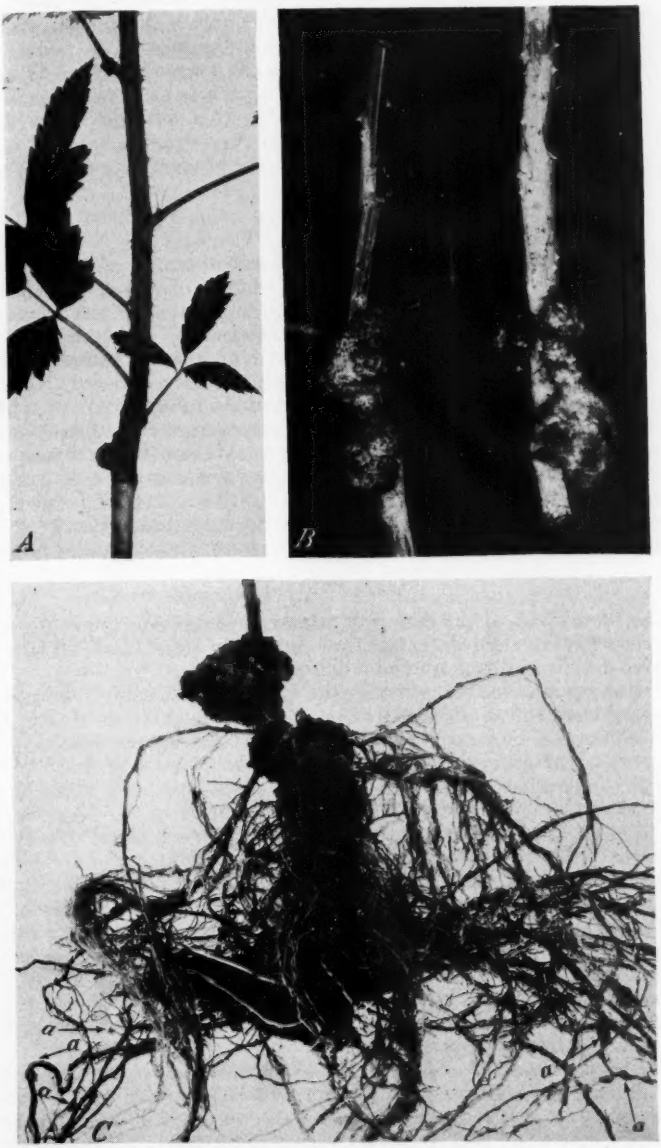

FIGURE 3.—Results of needle-puncture inoculations with the cane gall organism on Plum Farmer black raspberry plants: *A*, Gall symptoms on a petiole and stem of new cane growth 4 weeks after inolcuation; *B*, cane galls on current season stems 2 months after inoculation, with discoloration beginning to set in; *C*, cane galls on current-season growth about 11 weeks after inoculation. These galls measure over 1 inch in radial extension and the surface discoloration indicates a rapid state of deterioration. Small root galls (*a*) are also present.

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were produced by probing among the roots with a sharp tool. Several small galls of this character may be seen in figure 3, *C*, *a*.

When inoculations were made early in the season into fruiting canes, the incidence of infection was lower than when they were made into current-season growth. The reason for this was not ascertained. However, when infections were obtained there was a tendency for the galls to form ridges upward on the stem, the phenomenon so characteristic of infections occurring in the field.

HOST RANGE

Cane gall has been found in New York commercial plantings on black raspberries, purple raspberries, and blackberries, and when inoculated with the cane gall organism these plants and red raspberries also have been found susceptible to infection. On black raspberry canes 25 inoculations induced 25 galls with an average radial extension of 22 mm. after 6 weeks. Similarly on purple raspberry, red raspberry, and blackberry canes, 25 inoculations induced 25, 18, and 25 galls, respectively, with average radial extensions of 20, 4, and 18 mm. These results confirm a report (*18*) from Wisconsin for black raspberries. Although studies on host range are incomplete, it probably deserves mention that none of the plants thus far tested except the brambles have been found susceptible to infection. Pinckard (*13*) also failed to secure infection on tomato, beet, oleander, and olive.

LIFE HISTORY

The life history of the cane gall organism in relation to pathogenesis has received considerable attention especially from Banfield (*4*), but several details still remain to be determined.

The organism seems to enter the plant only through wounds or other injuries, for applications of bacteria to the surfaces of uninjured petioles, stems, and roots have always given negative results. The exact mode of entry in the field is unknown; however, root-feeding insects (*3*) or other insects that injure the plants, pruning wounds, and injuries to the underground parts produced in cultural operations, all would seem to afford adequate opportunity for bacterial invasion.

Banfield (*3*), in studying crown gall on red raspberry, observed that infections occurred only through injuries. A further significant fact observed was that injured tissue on the underground parts of red raspberry remain infection courts for as long as 7 weeks after injury. This slowness of wounds to heal favors the likelihood of invasion by all forms of wound pathogens, including the cane gall organism. Of perhaps equal significance is the delayed incubation period. This has been established for crown gall (*5*), but undoubtedly plays as significant a role in cane gall.

The incubation period (latent period of infection) based on inoculations during the summer months (June to September) was never less than about 2½ weeks and averaged nearer 3 weeks. This interval is somewhat longer than for the crown gall organism on raspberry as reported by Banfield (*3*). From two to four plants were inoculated at monthly intervals by needle punctures, each plant receiving an average of five inoculations into the growing canes. By judicious pruning, new growth was available each month. Inoculations were not attempted later than September, but for April and May the incubation periods averaged approximately 6 and 4 weeks.

The location of the casual organism in cane galls on black raspberry was thoroughly studied by Banfield (*4*), who found the bacteria principally between the cell walls but ramifying throughout all regions of the gall in the form of zoogloeal strands which dissolved away the middle lamella of the cell walls for channels. The gall initials found beneath the phelloderm of fruiting canes in early spring appear to be induced by hyperplasia [4] of the pericycle and phloem ray cells. From the time of the first microscopic appearance of the gall to its maturity, bacterial pockets are of common occurrence in the meristematic areas. Apparently they result from successive lysis and collapse of cells in contact with the bacterial strands; the cells just beyond the bacterial strands divide and those farther removed are not stimulated. As the galls near maturity, cell division becomes progressively more feeble and tissue degeneration more general. It is an interesting fact that the cell walls which are composed largely of cellulose appear not to be dissolved by the bacteria.

The bacteria may be discharged (*4*) from the cavities or pockets in the gall before disintegration sets in. Discharge takes place by way of intercellular channels to the surface which are presumed to have been occupied by bacterial strands.

The cane gall organism was found only in gall tissue when current season stems were inoculated at two widely separated points. Galls 2½ months old produced near the apex or the base of current season canes when they were about 12 inches long were employed. In one case isolations were attempted from the fresh galls and from the apparently healthy stem between these galls cut into four 3-inch pieces. Bacteria were obtained only from the gall tissue. In another case, the galls and stem pieces were immersed in calcium hypochlorite solution [5] for 20 minutes to reduce surface contaminants. Subsequently the galls were passed through sterile distilled water seven times, changing at hourly intervals. Platings were made from the washings, employing potato-mannitol agar and aniline blue selective medium. The results indicate that the bacteria were limited to the immediate gall region, that they had not migrated in current season canes, and that they were freely given off at the gall surface when immersed in water. Only one washing, that immediately following immersion in calcium hypochlorite solution, failed to yield bacteria in abundance from the uninjured galls, and this was undoubtedly due to the residual effects of the disinfectant.

In a similar experiment on fruiting canes the bacteria were obtained from the galls and also from the stem tissue for a short distance (up to 10 mm.) immediately above the galls, a fact which suggests that the fruiting canes must differ in some respects from current-season canes since they afford passage for the bacteria.

Longevity experiments seem to indicate that the period of survival of the cane gall bacterium in soil is much shorter than the periods of a year or more found earlier for the hairy root (*8*) and crown gall (*3*) organisms. Steamed and unsteamed soil were artificially infested with cane gall bacteria and stored either out of doors or in the greenhouse in early October. Isolations were attempted at monthly intervals, the isolation technique of Hildebrand (*8*) being used. The soil samples

[4] Butler (*6*), who examined histologically cane galls on black raspberry received from Wisconsin, mentions the presence of both hypertrophy and "hyperplasy" in the older gall tissue.

[5] Consists of 10 gm. of calcium hypochlorite mixed with 140 cc. of distilled water, filtered, and used fresh at full strength.

consisted of duplicate ½-cubic-foot lots of moderately rich clay loam placed in wooden containers. The steaming treatment was carried on for 1 hour at 15 pounds pressure. The inoculum consisted of a 3-day-old growth of the bacteria on potato-mannitol agar. The maximum survival period found in these experiments approximated 6 months in soil stored outdoors and 4 months in soil stored in the greenhouse, and in each instance the length of survival was about 1 month longer in steamed than in unsteamed soil. The relatively short period of survival of the cane gall bacteria in soil would seem to point to some other mode of overwintering, such as in the plant itself, ut the solution of this problem remains for the future.

THE CAUSAL ORGANISM

The methods given in Pure Culture Study of Bacteria as of 1933–36 were followed except as noted (*15*, *16*). In the studies on physiology, the chief reliance was placed on results given by Pinckard (*13*), whose work on this organism was generally repeated and confirmed.

GROWTH IN CULTURE

When isolations were made from young 3-week-old galls, minute white colonies were visible on the surface of potato-mannitol, potato-dextrose, or yeast-extract-dextrose agar after 3 to 5 days, and after 2 to 3 days when isolations were made from the organism in culture. At the end of 10 days at 24° C. the colonies were circular, smooth, entire, and raised, with the largest not much more than 4 mm. in diameter when the number of colonies to the plate approximated 100. Submerged colonies were disk-shaped (double convex) and approached half the size of surface colonies.

When aniline-blue agar was employed, it was not uncommon for many of the colonies to take up the dye, but tbis did not prove to be of positive diagnostic value.[6] The aniline-blue medium did serve to distinguish the cane gall from the crown gall and hairy root organisms. When streaks were made on the surface of this agar, the cane gall organism, like the crown gall organism, took up the dye, but the hairy root organism did not. Although both absorbed the dye, the cane gall organism made much less growth than the crown gall organism and produced a narrow, thin, almost flat streak in contrast to the broad raised streaks produced by the other two organisms.

In nutrient-broth cultures of the cane gall organism a uniform moderate turbidity was produced in about 2 days, but no pellicle was formed. On potato-mannitol-agar slants a moderate, filiform, glistening white growth developed along the streak in 48 hours. The edges were entire and the consistency of the growth was at first watery to butyrous. Upon aging the growth became a dull creamy white, spread irregularly, flattened, and changed to a tough leather consistency.

The cane gall organism grows slowly as compared with other plant pathogens, an observation also made by Pinckard (*13*). In a study of growth rates (*9*), it was demonstrated that this organism grew more

[6] Because both crown gall and cane gall bacteria take up aniline-blue dye readily when streaked on aniline-blue agar, it was thought that these bacteria might possibly take up the dye when isolations were attempted with poured plates. However, after repeated experiment, it was found that when dilution plates were poured, from none to many colonies took up the dye and the organisms from dyed as well as from undyed colonies were pathogenic. Hendrickson et al. (7) also noted that crown gall bacteria have strains which do not absorb dye. This phase of the work deserves further study.

slowly than any of 11 species of plant pathogens, having a minimum generation time of 155 minutes.

MORPHOLOGY

When grown in culture media, the cane-gall organism is a rod with rounded ends usually appearing singly or in pairs but sometimes occurring as short chains. Banfield (*4*), however, observed that when present in host tissue these bacteria are always grouped in chains or in masses and do not occur singly. After having grown on potato-mannitol-agar slants for 48 hours at 24° C. the cells were found to have a mean size of 1.72 μ by 0.64 μ when mounted with congo red negative stain. A repetition of the measurements of 6 of the 13 cultures taken at random from the collection gave a mean size of 1.72 μ by 0.63 μ. For each culture, 100 individual cells were measured.

The organism was motile in several liquid and solid media. Presumptive evidence for motility was obtained when turbidity was observed at considerable distances from the center in tubes of soft potato-mannitol agar (0.3 percent agar) which had been seeded by stab puncture. Following the isolation of single cells in potato-mannitol agar and nutrient broth, the appearance and progress of motility of the progeny cultures was more pronounced in broth than on agar, and on the fourth, fifth, and sixth days after isolation into microculture than earlier.

Polar flagella were demonstrated by means of Gray's and Casares-Gil's flagella stains. The former proved better but still not entirely satisfactory because of difficulty in getting the flagella to take up the dye. Several long flagella were commonly observed near one of the poles in a subpolar position. Only rather rarely were they observed at both poles and in these cases it is possible that the cells were nearing the fission point. Some of the flagella were 10 times as long as the individual cells.

PHYSIOLOGY

The work of Pinckard (*13*) on the utilization of various sources of carbon and nitrogen [7] was repeated and verified by the use of his mineral salt yeast-extract basal medium. Attempts to use the Society of American Bacteriologists' basal medium or to substitute simpler substances, such as *d*-glutamic acid, asparagine, inositol, ascorbic acid, and thiamin for yeast extract, were unsuccessful. All tests were made in quadruplicate. Acid was produced from arabinose, xylose, rhamnose, fructose, mannose, galactose, glucose, lactose, and erythritol. An alkaline reaction appeared with melezitose, starch, inulin, pectin, lactositol, calcium gluconate, formic acid, acetic acid, propionic acid, glycollic acid, malonic acid, succinic acid, tartaric acid, malic acid, and yeast extract only. Cellulose was not fermented.

The cane gall organism appears to be able to use most readily the more complex nitrogen compounds such as ferric ammonium citrate, uric acid, oxamide, succinimide, *l*-asparagune, *l*-tyrosine, *l*-cystine, *d*-glutamic acid, and yeast extract.

Gelatin was not liquefied. On litmus milk a slight serum zone, pink color, acid and curd were produced. Nitrates were not utilized

[7] The writer retested all of Pinckard's carbon sources except aesculin, phloridzin, and calcium gluconate but did not make any determinations on titratable acidity.

and nitrites not produced in the synthetic nitrate medium listed in the Manual of Methods of the Society of American Bacteriologists (*14*). When grown in Bacto-nutrient broth there was a slight test for ammonia (Hansen's method *16, p. 12*). In Bacto-tryptone broth negative tests were obtained for hydrogen sulphide production with ZoBell's method (*15, p. 11*) and for indole with Gnezda's technique (*15, p. 10*). Starch was not hydrolyzed and casein not digested. In agar shake cultures, only slight turbidity developed below the surface, placing the organism somewhere between an aerobe and a facultative anaerobe as to oxygen requirements. No apparent gas was produced. The organism when grown in nutrient broth had a thermal death point of about 56° C.

TAXONOMY

The cane gall organism is considered to be a member of the genus *Phytomonas* and the name *Phytomonas rubi* n. sp. is proposed for it. Synonyms (according to the classifications used by plant pathologists) would be *Bacterium rubi* n. sp. and *Pseudomonas rubi* n. sp.

TECHNICAL DESCRIPTION

Phytomonas rubi n. sp.

Small, Gram-negative, non-acid-fast rod (1.72 μ by 0.64 μ) with rounded ends, chiefly occurring singly and in pairs, occasionally in short chains in culture and in chains and masses but not singly in host tissue. Weak facultative anaerobe with optimum growth at 27° C.; thermal death point about 56° C.; motile by subpolar flagella; spores not formed.

On potato-mannitol-agar slants growth slow, moderate, filiform, white to creamy-white, with butyrous consistency later becoming leathery. Uniform clouding of bouillon cultures in 36 to 48 hours. Gelatin not liquefied; starch not hydrolyzed; nitrates not reduced but slight ammonia produced in nutrient broth; hydrogen sulfide and indole not formed. Acid produced in milk. Acid but no apparent gas from arabinose, xylose, rhamnose, fructose, mannose, galactose, glucose, lactose, erythritol. Alkali but no apparent gas from melezitose, starch, inulin, pectin, lactositol, calcium gluconate, formic acid, acetic acid, propionic acid, glycollic acid, malonic acid, succinic acid, tartaric acid, malic acid, and yeast extract.

Although it also adsorbs dye, it is readily distinguished from crown gall organism when streaked on aniline-blue agar; slow-growing with minimum generation time of about 155 minutes.

Pathogenic on black and purple raspberries, blackberries, and, to much lesser extent, on red raspberry.

DISCUSSION

Cane gall has some things in common with crown gall, especially in gross symptomatalogy and in certain phases of the life history of the causal organisms. *Phytomonas rubi* is distinctly different from *P. tumefaciens* in its physiology and pathogenicity, including growth character on artificial media, a preference for complex nitrogen sources in its metabolism, and a restricted host range. These and other characteristics summarized in table 1 constitute a basis for giving it specific rank. The characteristic of motility by subpolar flagella may have significance in determining its taxonomic position, but for the present it is placed in the genus *Phytomonas*.

At present, cane gall is of minor importance in New York, thanks largely to the efficiency of the inspection service and extension work. Control measures that are adequate for crown gall are easily adequate for cane gall. One reason may be that cane gall generally occurs upon

the visible parts of the canes, which facilitates eradication; another that the cane gall organism apparently is less able than the crown gall organism to survive in soil. Obviously this latter observation needs further study. Prof. L. M. Cooley, formerly at the Geneva (N. Y.) Experiment Station, who cooperated with the writer in the field in the course of these observations on raspberry diseases, noted a progressive decline in cane gall in western New York plantings, and at the present time his successor, Dr. R. F. Suit, has infrequently encountered it in commercial plantings in areas where it was formerly present.

TABLE 1.—*Differential characteristics of the cane gall organism (Phytomonas rubi n. sp.), and the crown gall organism (P. tumefaciens)*

Differential characters	Cane gall organism	Crown gall organism
Symptoms on naturally infected plants.	Galls found at and above soil level.	Galls found at and below soil level.
	Galls discolor and decompose rapidly; ordinarily unable to isolate organism after July 1.	Galls, while subject to decomposition, may persist throughout season.
	Long gall ridges or galls distributed indiscriminately on canes.	Galls generally occur individually.
	Bacteria migrate up through outer tissue of cane.	Bacteria unable to migrate up the cane in same manner.
Symptoms on artificially infected plants.	Very small galls on roots.	Large galls on roots.
	Small galls below soil level on canes.	Large galls at and below soil level on canes.
	Large galls above ground.	Small galls above ground.
Longevity of organism in soil	Few months.	1 to 3 years.
Host range	Limited genus *Rubus*.	Very wide; includes many plant families.
Minimal incubation period	2½ to 3 weeks.	10 days to 2 weeks.
Motility of bacteria	Motile.	Doubtful motility in many cases.
Stability of bacteria in culture.	Unstable; pathogenicity frequently lost within a year.	Very stable; pathogenicity retained for 10 years and longer.
Minimal generation time of bacteria.	155 minutes.	78 minutes.
Growth in:		
Nutrient-dextrose broth	Weak growth, slight or no pellicle.	Strong growth, heavy pellicle.
Potato-mannitol agar	Thin leathery growth with time.	Abundant growth, watery to butyrous.
Nitrogen metabolism	Unable to use potassium nitrate, ammonium nitrate, ammonium sulfate, potassium nitrite, urea, dicyandiamide and acetamide.	Ammonium nitrate, ammonium sulfate, potassium nitrite, urea, dicyandiamide, and acetamide support excellent growth.
Reaction on litmus milk	Slight serum zone.	Heavy serum zone.
	Pink color.	Grayish brown.
	Acid.	Neutral.

SUMMARY

Cane gall, a rather widely distributed but economically relatively unimportant disease of *Rubus* spp., has been investigated and a description given of its symptoms. The characteristic beading and elongate gall ridges on the above-ground canes are in marked contrast to crown gall which ordinarily occurs at or below ground level. The causal organism was readily isolated from young galls, its pathogenicity was proved, and it was studied in detail. The name proposed for the pathogen is *Phytomonas rubi* n. sp. The organism was found to be pathogenic on black raspberry, purple raspberry, blackberry, and red raspberry, but only weakly so on the last-named species upon which it rarely occurs in the field.

LITERATURE CITED

(1) BAILEY, L. H.
1895. BLACKBERRIES. N. Y. (Cornell) Agr. Expt. Sta. Bul. 99: 413–438, illus.

(2) BANFIELD, W. M.
1930. CANE GALL OF BLACK RASPBERRY. (Abstract) Phytopathology 20: 123–124.

(3) BANFIELD, W. M.
1934. LIFE HISTORY OF THE CROWN-GALL ORGANISM IN RELATION TO ITS PATHOGENESIS ON THE RED RASPBERRY. Jour. Agr. Res. 48: 761–787, illus.
(4) ———
1935. STUDIES ON CELLULAR PATHOLOGY. I. EFFECTS OF CANE GALL BACTERIA UPON GALL TISSUE CELLS OF THE BLACK RASPBERRY. Bot. Gaz. 97: 193–239, illus.
(5) ——— and MANDENBERG, E. C.
1935. IMPORTANCE OF SANITATION IN CONTROLLING CROWN GALL OF THE RED RASPBERRY. (Abstract) Phytopathology 25: 5–6.
(6) BUTLER, E. J.
1930. SOME ASPECTS OF THE MORBID ANATOMY OF PLANTS. Ann. Appl. Biol. 17: [175]–212, illus.
(7) HENDRICKSON, A. A., BALDWIN, I. L., and RIKER, A. J.
1934. STUDIES ON CERTAIN PHYSIOLOGICAL CHARACTERS OF PHYTOMONAS TUMEFACIENS, PHYTOMONAS RHIZOGENES AND BACILLUS RADIOBACTER. Jour. Bact. 28: 597–618, illus.
(8) HILDEBRAND, E. M.
1934. LIFE HISTORY OF THE HAIRY-ROOT ORGANISM IN RELATION TO ITS PATHOGENESIS ON NURSERY APPLE TREES. Jour. Agr. Res. 48: 857–885, illus.
(9) ———
1938. GROWTH RATES OF PHYTOPATHOGENIC BACTERIA. Jour. Bact. 35: 487–492.
(10) ———
1938. TECHNIQUES FOR THE ISOLATION OF SINGLE MICROORGANISMS. Bot. Rev. 4: 627–664.
(11) IVANOFF, S. S.
1933. STEWART'S WILT DISEASE OF CORN, WITH EMPHASIS ON THE LIFE HISTORY OF PHYTOMONAS STEWARTI IN RELATION TO PATHOGENESIS. Jour. Agr. Res. 47: 749–770, illus.
(12) PATEL, M. K.
1926. AN IMPROVED METHOD OF ISOLATING PSEUDOMONAS TUMEFACIENS SM. AND TOWN. Phytopathology 16: 577.
(13) PINCKARD, J. A.
1935. PHYSIOLOGICAL STUDIES OF SEVERAL PATHOGENIC BACTERIA THAT INDUCE CELL STIMULATION IN PLANTS. Jour. Agr. Res. 50: 933–952, illus.
(14) SOCIETY OF AMERICAN BACTERIOLOGISTS, COMMITTEE ON BACTERIOLOGICAL TECHNIC.
1936. MANUAL OF METHODS FOR PURE CULTURE STUDY OF BACTERIA. 5 v., illus. Geneva, N. Y.
(15) ———
1937. ROUTINE TESTS FOR THE DESCRIPTIVE CHART: MORPHOLOGICAL AND BIOCHEMICAL. Pure Cult. Study Bact., v. 5, No. 3, 20 pp., illus.
(16) ———
1940. FURTHER BIOCHEMICAL METHODS. Pure Cult. Study Bact., v. 8, No. 1, 16 pp., illus.
(17) SORAUER, PAUL.
1914–22. MANUAL OF PLANT DISEASES. VOLUME 1. NONPARASITIC DISEASES. 908 pp., illus. Berlin.
(18) WISCONSIN AGRICULTURAL EXPERIMENT STATION.
1930. NEW EVIDENCE SECURED ON BLACK RASPBERRY CANE GALL. Wis. Agr. Expt. Sta. Bul. 410: 109–110, illus.
(19) WULFF, THORILD.
1908. STUDIEN ÜBER HETEROPLASTISCHE GEWEBEWUCHERUNGEN AM HIMBEER UND AM STACHELBEERSTRAUCH. Arkiv. för Bot Bd. 7, No. 14, 32 pp., illus.
(20) ———
1909. WEITERE STUDIEN ÜBER DIE KALLUSKRANKHEIT DES HIMBEERSTRAUCHES. Arkiv. för Bot., Bd. 8, No. 15, 6 pp.
(21) ZUNDEL, GEORGE L.
1934. RASPBERRY DISEASES. Pa. State Col. Ext. Cir. 133, 20 pp., illus. [Revised.]

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